```
* * * * * * * * * *  Welcome to STN International  * * * * * * * * * *

 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09  CA/CAplus records now contain indexing from 1907 to the
                  present
 NEWS  4  DEC 08  INPADOC: Legal Status data reloaded
 NEWS  5  SEP 29  DISSABS now available on STN
 NEWS  6  OCT 10  PCTFULL: Two new display fields added
 NEWS  7  OCT 21  BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28  BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24  MSDS-CCOHS file reloaded
 NEWS 10  DEC 08  CABA reloaded with left truncation
 NEWS 11  DEC 08  IMS file names changed
 NEWS 12  DEC 09  Experimental property data collected by CAS now available
                  in REGISTRY
 NEWS 13  DEC 09  STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17  DGENE: Two new display fields added
 NEWS 15  DEC 18  BIOTECHNO no longer updated
 NEWS 16  DEC 19  CROPU no longer updated; subscriber discount no longer
                  available
 NEWS 17  DEC 22  Additional INPI reactions and pre-1907 documents added to CAS
                  databases
 NEWS 18  DEC 22  IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22  ABI-INFORM now available on STN
 NEWS 20  JAN 27  Source of Registration (SR) information in REGISTRY updated
                  and searchable
 NEWS 21  JAN 27  A new search aid, the Company Name Thesaurus, available in
                  CA/CAplus
 NEWS 22  FEB 05  German (DE) application and patent publication number format
                  changes
 NEWS 23  MAR 03  MEDLINE and LMEDLINE reloaded
 NEWS 24  MAR 03  MEDLINE file segment of TOXCENTER reloaded
 NEWS 25  MAR 03  FRANCEPAT now available on STN

 NEWS EXPRESS  MARCH 5 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 3 MARCH 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
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  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
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CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 9 Mar 2004 (20040309/PD)
FILE LAST UPDATED: 9 Mar 2004 (20040309/ED)
HIGHEST GRANTED PATENT NUMBER: US6704933
HIGHEST APPLICATION PUBLICATION NUMBER: US2004045070
CA INDEXING IS CURRENT THROUGH 9 Mar 2004 (20040309/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 9 Mar 2004 (20040309/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATALL. Type FILE USPATALL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATALL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

```
=> e sanders david a/in
E1            1     SANDERS DAVE C/IN
E2           16     SANDERS DAVID/IN
E3            1 --> SANDERS DAVID A/IN
E4            1     SANDERS DAVID ALBERT/IN
E5            6     SANDERS DAVID C/IN
E6            1     SANDERS DAVID D/IN
E7           30     SANDERS DAVID E/IN
E8            1     SANDERS DAVID EDWIN/IN
E9            1     SANDERS DAVID F/IN
E10           1     SANDERS DAVID G/IN
E11           4     SANDERS DAVID H/IN
E12          15     SANDERS DAVID J/IN

=> s e3 or e4
              1 "SANDERS DAVID A"/IN
              1 "SANDERS DAVID ALBERT"/IN
L1            2 "SANDERS DAVID A"/IN OR "SANDERS DAVID ALBERT"/IN

=> d l1,ti,1-3

L1   ANSWER 1 OF 2  USPATFULL on STN
TI     Intrinsically safe communication systems

L1   ANSWER 2 OF 2  USPATFULL on STN
TI     Liquid crystal display device and method of making


=> e fischbach michael a/in
E1            1     FISCHBACH MAX/IN
E2            2     FISCHBACH MELVYN ROBERT/IN
E3            0 --> FISCHBACH MICHAEL A/IN
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E5            1      FISCHBACH PAUL/IN
E6            1      FISCHBACH PHILLIP C/IN
E7            1      FISCHBACH PIERRE/IN
E8            1      FISCHBACH RAINER W/IN
E9            8      FISCHBACH REINHARD/IN
E10           3      FISCHBACH RODERICK C/IN
E11           1      FISCHBACH RONALD/IN
E12           3      FISCHBACH STEFAN/IN

=> s e4
L2            1 "FISCHBACH MICHEL"/IN

=> d l2,ti

L2   ANSWER 1 OF 1  USPATFULL on STN
TI     Method for the inducible production of proteins in genetically modified
       eukaryotic host-cells multiplied in vivo


=> d l2,cbib,ab

L2   ANSWER 1 OF 1  USPATFULL on STN
97:24899 Method for the inducible production of proteins in genetically
    modified eukaryotic host-cells multiplied in vivo.
    Bromley, Peter, Chene-Bougertes, Switzerland
    Dreano, Michel, Vessy, Switzerland
    Fischbach, Michel, Quetigny, France
    Fouillet, Xavier, Collonges s/Saleve, France
    Padieu, Prudent, Dijon, France
    Voellmy, Richard, Miami, FL, United States
    Rothwell Properties Limited, Douglas, Isle of Man (non-U.S. corporation)
    US 5614381 19970325
    APPLICATION: US 1995-421277  19950413 (8)
    PRIORITY: EP 1986-810455  19861015
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     In the production of proteins of biological interest by means of a
       stress inducible gene expression unit/eukaryotic host cell system, the
       transformed cell lines are multiplicated by tumour growing in
       immunodefficient warm-blooded animals, after which the multiplicated
       cells are cultured in vitro and subjected to stress, whereby expression
       occurs in high yield. In vivo multiplication rates of 10^5 -10^6
       the innoculated quantity/2 weeks are reported without any loss of the
       latent inducible expression capacity.


=> e kuhn richard j/in
E1           18      KUHN REINHARD/IN
E2            3      KUHN RICHARD B/IN
E3            2 --> KUHN RICHARD J/IN
E4            1      KUHN RICHARD T/IN
E5            5      KUHN ROBERT/IN
E6            1      KUHN ROBERT A/IN
E7            2      KUHN ROBERT F/IN
E8            2      KUHN ROBERT H/IN
E9            1      KUHN ROBERT J/IN
E10           5      KUHN ROBERT L/IN
E11           1      KUHN ROBERT M/IN
E12           1      KUHN ROBERT M JR/IN

=> s e3
L3            2 "KUHN RICHARD J"/IN

=> d l3,ti,1-2
```

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TI     Channel expander

L3   ANSWER 2 OF 2  USPATFULL on STN
TI     Visual receiver


=> e jeffers scott a/in
E1            1     JEFFERS ROBERT P/IN
E2            3     JEFFERS SCOTT/IN
E3            1 --> JEFFERS SCOTT A/IN
E4            2     JEFFERS SCOTT E/IN
E5            1     JEFFERS SR WALTER L/IN
E6            1     JEFFERS STEVEN N/IN
E7            1     JEFFERS TERRY E/IN
E8            1     JEFFERS THOMAS H/IN
E9            2     JEFFERS THOMAS K/IN
E10           5     JEFFERS WILLIAM/IN
E11           1     JEFFERS WILLIAM D/IN
E12           1     JEFFERS WILLIAM O/IN

=> s e3
L4            1 "JEFFERS SCOTT A"/IN

=> d l4,ti

L4   ANSWER 1 OF 1  USPATFULL on STN
TI     Controlled release polymeric compositions of bone growth promoting
       compounds


=> e north cynthia l/iin
'IIN' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'USPATFULL'
The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
SFIELDS at an arrow prompt (=>).

=> e north cynthia l/in
E1            3     NORTH CHRISTOPHER/IN
E2            1     NORTH CHRISTOPHER JAMES GUILDFORD/IN
E3            0 --> NORTH CYNTHIA L/IN
E4            1     NORTH DANIEL A/IN
E5            1     NORTH DANIEL D/IN
E6            1     NORTH DARRYL L/IN
E7            1     NORTH DAVE/IN
E8            1     NORTH DAVID/IN
E9            2     NORTH DAVID C/IN
E10           1     NORTH DAVID D/IN
E11           1     NORTH DAVID G/IN
E12           1     NORTH DAVID M/IN

=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     5.89       6.10

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004

 FILE LAST UPDATED: 9 MAR 2004 (20040309/UP).  FILE COVERS 1953 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
```

description of changes:

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> e sanders d a/au
E1            1     SANDERS COURTNEY/AU
E2          188     SANDERS D/AU
E3           57 --> SANDERS D A/AU
E4          110     SANDERS D B/AU
E5            1     SANDERS D BRADFORD/AU
E6           14     SANDERS D C/AU
E7            3     SANDERS D D/AU
E8           48     SANDERS D E/AU
E9           19     SANDERS D G/AU
E10           8     SANDERS D H/AU
E11          65     SANDERS D J/AU
E12           1     SANDERS D K/AU

=> s e2 or e3
            188 "SANDERS D"/AU
             57 "SANDERS D A"/AU
L5          245 "SANDERS D"/AU OR "SANDERS D A"/AU

=> s l5 and (retrovir? or expression vector? or ross river virus or pseudotyp?)
          31891 RETROVIR?
         610567 EXPRESSION
          99135 VECTOR?
          11851 EXPRESSION VECTOR?
                  (EXPRESSION(W)VECTOR?)
           1895 ROSS
           9668 RIVER
         369440 VIRUS
            269 ROSS RIVER VIRUS
                  (ROSS(W)RIVER(W)VIRUS)
           1062 PSEUDOTYP?
L6            6 L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS OR
                  PSEUDOTYP?)

=> d l6,ti,1-6

L6   ANSWER 1 OF 6     MEDLINE on STN
TI   Fv-4: identification of the defect in Env and the mechanism of resistance
     to ecotropic murine leukemia virus.

L6   ANSWER 2 OF 6     MEDLINE on STN
TI   Ross River virus glycoprotein-pseudotyped retroviruses and
     stable cell lines for their production.

L6   ANSWER 3 OF 6     MEDLINE on STN
TI   Plasmid transfection and retroviral transduction of porcine muscle cells
     for cell-mediated gene transfer.

L6   ANSWER 4 OF 6     MEDLINE on STN
TI   Localization of the labile disulfide bond between SU and TM of the murine
     leukemia virus envelope protein complex to a highly conserved CWLC motif
     in SU that resembles the active-site sequence of thiol-disulfide exchange
     enzymes.

L6   ANSWER 5 OF 6     MEDLINE on STN
TI   Two distinct oncornaviruses harbor an intracytoplasmic tyrosine-based
     basolateral targeting signal in their viral envelope glycoprotein.

L6   ANSWER 6 OF 6     MEDLINE on STN
TI   Expression of functional bovine cholesterol side chain cleavage cytochrome
     P450 (P450scc) in Escherichia coli.
```

=> d l6,cbib,ab,1-3

L6 ANSWER 1 OF 6 MEDLINE on STN
2001556016. PubMed ID: 11602766. Fv-4: identification of the defect in Env and the mechanism of resistance to ecotropic murine leukemia virus. Taylor G M; Gao Y; **Sanders D A**. (Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392, USA.) Journal of virology, (2001 Nov) 75 (22) 11244-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Mice expressing the Fv-4 gene are resistant to infection by ecotropic murine leukemia viruses (MuLVs). The Fv-4 gene encodes an envelope (Env) protein whose putative receptor-binding domain resembles that of ecotropic MuLV Env protein. Resistance to ecotropic MuLVs appears to result from viral interference involving binding of the endogenously expressed Fv-4 env-encoded protein to the ecotropic receptor, although the immune system also plays a role in resistance. The Fv-4 env-encoded protein is processed normally and can be incorporated into virus particles but is unable to promote viral entry. Among the many sequence variations between the transmembrane (TM) subunit of the Fv-4 env-encoded protein and the TM subunits of other MuLV Env proteins, there is a substitution of an arginine residue in the Fv-4 env-encoded protein for a glycine residue (gly-491 in Moloney MuLV Env) that is otherwise conserved in all of the other MuLVs. This residue is present in the MuLV TM fusion peptide sequence. In this study, gly-491 of Moloney MuLV Env has been replaced with other residues and a mutant Env bearing a substitution for gly-487 was also created. G491R recapitulates the Fv-4 Env phenotype in cell culture, indicating that this substitution is sufficient for creation of an Env protein that can establish the interference-mediated resistance to ecotropic viruses produced by the Fv-4 gene. Analysis of the mutant MuLV Env proteins also has implications for an understanding of the role of conserved glycine residues in fusion peptides and for the engineering of organismal resistance to **retroviruses**.

L6 ANSWER 2 OF 6 MEDLINE on STN
2001196568. PubMed ID: 11222688. **Ross River virus** glycoprotein-**pseudotyped retroviruses** and stable cell lines for their production. Sharkey C M; North C L; Kuhn R J; **Sanders D A**. (Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392, USA.) Journal of virology, (2001 Mar) 75 (6) 2653-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Pseudotyped retroviruses** have important applications as vectors for gene transfer and gene therapy and as tools for the study of viral glycoprotein function. Recombinant Moloney murine leukemia virus (Mo-MuLV)-based **retrovirus** particles efficiently incorporate the glycoproteins of the alphavirus **Ross River virus** (RRV) and utilize them for entry into cells. Stable cell lines that produce the RRV glycoprotein-**pseudotyped retroviruses** for prolonged periods of time have been constructed. The **pseudotyped** viruses have a broadened host range, can be concentrated to high titer, and mediate stable transduction of genes into cells. The RRV glycoprotein-**pseudotyped retroviruses** and the cells that produce them have been employed to demonstrate that RRV glycoprotein-mediated viral entry occurs through endocytosis and that membrane fusion requires acidic pH. Alphavirus glycoprotein-**pseudotyped retroviruses** have significant advantages as reagents for the study of the biochemistry and prevention of alphavirus entry and as preferred vectors for stable gene transfer and gene therapy protocols.

L6 ANSWER 3 OF 6 MEDLINE on STN
2000244766. PubMed ID: 10784180. Plasmid transfection and **retroviral** transduction of porcine muscle cells for cell-mediated gene transfer. Blanton J R Jr; Bidwell C A; **Sanders D A**; Sharkey C M; McFarland D C; Gerrard D E; Grant A L. (Department of Animal Sciences, Purdue University, West Lafayette, IN 47907, USA.) Journal of animal science, (2000 Apr) 78

States. Language: English.

AB Cell-mediated gene transfer is a potential tool for studying muscle growth, but efficient genetic manipulation and implantation strategies have not been developed for pigs. The objectives of the present study were to determine methods for transient and stable incorporation of reporter genes into porcine muscle cells and to investigate their use for cell-mediated gene transfer in pigs. Porcine myoblasts and fibroblasts were isolated from muscle of 2-wk-old male pigs. Myogenic cell lines were identified using muscle-specific monoclonal antibodies, myotube fusion assays, and the presence of muscle-specific markers (MyoD and desmin). Four commercial cationic liposomes (lipofectAMINE, lipofectin, cellFECTIN, and DMRIE-C) were tested at different DNA:lipid ratios for their ability to transfect myoblasts and fibroblasts transiently with a luciferase reporter plasmid. LipofectAMINE resulted in the greatest ($P < .01$) transient luciferase activity for both cell types. Electroporation of cells for transient transfection resulted in less luciferase activity than cationic transfection. Stable transfections were conducted using a green fluorescence protein (GFP) reporter plasmid containing the neomycin resistance gene. LipofectAMINE transfection resulted in stable GFP expression in 1:16,000 myoblasts and 1:33,000 fibroblasts. Stable electroporation resulted in efficiencies that were significantly lower than established with cationic liposomes. Porcine cells were transduced with GFP using vesicular stomatitis virus glycoprotein G **pseudotyped retrovirus** and resulted in efficiencies of 1:1.2 for myoblasts and 1:1.1 for fibroblasts. These results show that cationic liposomes are superior to electroporation for transfection, but **retroviral** transduction produced stable reporter gene expression in > 80% of porcine muscle cells. Transduced GFP-positive cells were separated from GFP-negative cells by fluorescence-activated cell sorting and implanted into 2-wk-old male pigs. On d 4, implanted muscles were removed and subjected to immunodetection of GFP protein. Fibroblast implantation resulted in limited GFP expression within muscle, whereas myoblast implantation resulted in GFP within muscle fibers. This suggests that cell-mediated gene transfer is possible in porcine muscle and may be useful as an approach for studying muscle growth in pigs.

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=> d his

     (FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
                E SANDERS DAVID A/IN
L1            2 S E3 OR E4
                E FISCHBACH MICHAEL A/IN
L2            1 S E4
                E KUHN RICHARD J/IN
L3            2 S E3
                E JEFFERS SCOTT A/IN
L4            1 S E3
                E NORTH CYNTHIA L/IN

     FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
                E SANDERS D A/AU
L5          245 S E2 OR E3
L6            6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O

=> e fischbach m a/au
E1            3     FISCHBACH LORI A/AU
E2          134     FISCHBACH M/AU
E3            0 --> FISCHBACH M A/AU
E4            2     FISCHBACH M R/AU
E5            2     FISCHBACH M S/AU
E6            6     FISCHBACH M W/AU
E7            1     FISCHBACH MICHAEL/AU
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E9            8      FISCHBACH MICHEL/AU
E10           4      FISCHBACH N/AU
E11           3      FISCHBACH P/AU
E12          11      FISCHBACH P S/AU

=> s e2
L7          134 "FISCHBACH M"/AU

=> s l7 and (retrovir? or expression vector? or ross river virus or pseudotyp?)
         31891 RETROVIR?
        610567 EXPRESSION
         99135 VECTOR?
         11851 EXPRESSION VECTOR?
                 (EXPRESSION(W)VECTOR?)
          1895 ROSS
          9668 RIVER
        369440 VIRUS
           269 ROSS RIVER VIRUS
                 (ROSS(W)RIVER(W)VIRUS)
          1062 PSEUDOTYP?
L8           2 L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS OR
               PSEUDOTYP?)

=> s l8 not l6
L9           2 L8 NOT L6

=> d l9,ti,1-2

L9   ANSWER 1 OF 2     MEDLINE on STN
TI   Cloning of a human IgM autoantibody bearing a cross-reactive idiotype in a
     lambda expression vector: a new approach to studying autoantibodies.

L9   ANSWER 2 OF 2     MEDLINE on STN
TI   Genetic analysis of induction of anti-polyadenylic acid antibodies and
     xenotropic type-C viruses.


=> e kuhn r j/au
E1            1      KUHN R G/AU
E2            1      KUHN R H/AU
E3           95 --> KUHN R J/AU
E4            2      KUHN R L/AU
E5            6      KUHN R M/AU
E6            1      KUHN R P/AU
E7           45      KUHN R W/AU
E8            4      KUHN RAINER/AU
E9            8      KUHN RALF/AU
E10           1      KUHN RAPHAEL/AU
E11           1      KUHN REGINA J/AU
E12          21      KUHN REGNIER F/AU

=> s e3
L10          95 "KUHN R J"/AU

=> s l10 and (retrovir? or expression vector? or pseudotyp? or ross river virus)
         31891 RETROVIR?
        610567 EXPRESSION
         99135 VECTOR?
         11851 EXPRESSION VECTOR?
                 (EXPRESSION(W)VECTOR?)
          1062 PSEUDOTYP?
          1895 ROSS
          9668 RIVER
        369440 VIRUS
           269 ROSS RIVER VIRUS
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```
L11           9 L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROSS
                RIVER VIRUS)

=> d his

     (FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
                E SANDERS DAVID A/IN
L1            2 S E3 OR E4
                E FISCHBACH MICHAEL A/IN
L2            1 S E4
                E KUHN RICHARD J/IN
L3            2 S E3
                E JEFFERS SCOTT A/IN
L4            1 S E3
                E NORTH CYNTHIA L/IN

     FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
                E SANDERS D A/AU
L5          245 S E2 OR E3
L6            6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
                E FISCHBACH M A/AU
L7          134 S E2
L8            2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9            2 S L8 NOT L6
                E KUHN R J/AU
L10          95 S E3
L11           9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS

=> s l11 not (l6 or l9)
L12           8 L11 NOT (L6 OR L9)

=> d l12,ti,1-8

L12  ANSWER 1 OF 8      MEDLINE on STN
TI   An amino acid substitution in the coding region of the E2 glycoprotein
     adapts Ross River virus to utilize heparan sulfate as an attachment
     moiety.

L12  ANSWER 2 OF 8      MEDLINE on STN
TI   Locations of carbohydrate sites on alphavirus glycoproteins show that E1
     forms an icosahedral scaffold.

L12  ANSWER 3 OF 8      MEDLINE on STN
TI   In vitro assembly of alphavirus cores by using nucleocapsid protein
     expressed in Escherichia coli.

L12  ANSWER 4 OF 8      MEDLINE on STN
TI   Chimeric Sindbis-Ross River viruses to study interactions between
     alphavirus nonstructural and structural regions.

L12  ANSWER 5 OF 8      MEDLINE on STN
TI   Putative receptor binding sites on alphaviruses as visualized by
     cryoelectron microscopy.

L12  ANSWER 6 OF 8      MEDLINE on STN
TI   Nucleocapsid and glycoprotein organization in an enveloped virus.

L12  ANSWER 7 OF 8      MEDLINE on STN
TI   Nucleocapsid-glycoprotein interactions required for assembly of
     alphaviruses.

L12  ANSWER 8 OF 8      MEDLINE on STN
TI   Infectious RNA transcripts from Ross River virus cDNA clones and the
```

construction and characterization of defined chimeras with Sindbis virus.

=> d l12,cbib,ab,4

L12 ANSWER 4 OF 8 MEDLINE on STN
97048072. PubMed ID: 8892913. Chimeric Sindbis-Ross River viruses to study interactions between alphavirus nonstructural and structural regions.
Kuhn R J; Griffin D E; Owen K E; Niesters H G; Strauss J H. (Division of Biology, California Institute of Technology, Pasadena 91125, USA.)
Journal of virology, (1996 Nov) 70 (11) 7900-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Sindbis virus and **Ross River virus** are alphaviruses whose nonstructural proteins share 64% identity and whose structural proteins share 48% identity. Starting from full-length cDNA clones of both viruses, we have generated two reciprocal Sindbis-Ross River chimeric viruses in which the structural and nonstructural regions have been exchanged. These chimeric viruses replicate readily in several cell lines. Both chimeras grow more poorly than do the parental viruses, with the chimera containing Sindbis virus nonstructural proteins and **Ross River virus** structural proteins growing considerably better in both mosquito and Vero cell lines than the reciprocal chimera does. The reduction in replicative capacity in comparison with the parental viruses appears to result at least in part from a reduction in RNA synthesis, which suggests that the structural proteins or sequence elements within the structural region interact with the nonstructural proteins or sequence elements within the nonstructural region, that these interactions are required for efficient RNA replication, and that these interactions are suboptimal in the chimeras. The chimeras are able to infect mice, but their growth is attenuated. Western equine encephalitis virus, a virus widely distributed throughout the Americas, has been previously shown to have arisen by natural recombination between two distinct alphaviruses, but other naturally occurring recombinant alphaviruses have not been found. The present results suggest that most nonstructural/structural chimeras that might arise by natural recombination will be viable but that interactions between different regions of the genome, some of which were previously known but some of which remain unknown, limit the ability of such recombinants to become established.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS

```
=> e jeffers s a/au
E1            1     JEFFERS RUSSELL/AU
E2           31     JEFFERS S/AU
E3            0 --> JEFFERS S A/AU
E4            2     JEFFERS S L/AU
E5            2     JEFFERS SCOTT A/AU
E6           25     JEFFERS T A/AU
E7            1     JEFFERS T J/AU
E8           35     JEFFERS T K/AU
E9            1     JEFFERS T M/AU
E10           1     JEFFERS T R/AU
E11           2     JEFFERS V/AU
E12          27     JEFFERS W A/AU

=> s e2 or e5
             31 "JEFFERS S"/AU
              2 "JEFFERS SCOTT A"/AU
L13          33 "JEFFERS S"/AU OR "JEFFERS SCOTT A"/AU

=> s l13 and (retrovir? or expression vector? or pseudotyp? or ross river virus)
          31891 RETROVIR?
         610567 EXPRESSION
          99135 VECTOR?
          11851 EXPRESSION VECTOR?
                  (EXPRESSION(W)VECTOR?)
           1062 PSEUDOTYP?
           1895 ROSS
           9668 RIVER
         369440 VIRUS
            269 ROSS RIVER VIRUS
                  (ROSS(W)RIVER(W)VIRUS)
L14            2 L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROSS
                  RIVER VIRUS)

=> d l14,ti,1-2

L14  ANSWER 1 OF 2     MEDLINE on STN
TI   Lentivirus vectors pseudotyped with filoviral envelope glycoproteins
     transduce airway epithelia from the apical surface independently of folate
     receptor alpha.

L14  ANSWER 2 OF 2     MEDLINE on STN
TI   Covalent modifications of the ebola virus glycoprotein.


=> d l14,cbib,ab

L14  ANSWER 1 OF 2     MEDLINE on STN
2003199897.    PubMed ID: 12719583.   Lentivirus vectors pseudotyped with
     filoviral envelope glycoproteins transduce airway epithelia from the
     apical surface independently of folate receptor alpha. Sinn Patrick L;
     Hickey Melissa A; Staber Patrick D; Dylla Douglas E; Jeffers Scott A;
     Davidson Beverly L; Sanders David A; McCray Paul B Jr. (Program in Gene
     Therapy, Department of Pediatrics, University of Iowa College of Medicine,
     Iowa City, Iowa 52242, USA. ) Journal of virology, (2003 May) 77 (10)
     5902-10.  Journal code: 0113724. ISSN: 0022-538X. Pub. country: United
     States. Language: English.
AB   The practical application of gene therapy as a treatment for cystic
     fibrosis is limited by poor gene transfer efficiency with vectors applied
     to the apical surface of airway epithelia.  Recently, folate receptor
     alpha (FR alpha), a glycosylphosphatidylinositol-linked surface protein,
     was reported to be a cellular receptor for the filoviruses.  We found that
     polarized human airway epithelia expressed abundant FR alpha on their
     apical surface.  In an attempt to target these apical receptors, we
```

pseudotyped feline immunodeficiency virus (FIV)-based vectors by using envelope glycoproteins (GPs) from the filoviruses Marburg virus and Ebola virus. Importantly, primary cultures of well-differentiated human airway epithelia were transduced when filovirus GP-**pseudotyped** FIV was applied to the apical surface. Furthermore, by deleting a heavily O-glycosylated extracellular domain of the Ebola GP, we improved the titer of concentrated vector severalfold. To investigate the folate receptor dependence of gene transfer with the filovirus **pseudotypes**, we compared gene transfer efficiency in immortalized airway epithelium cell lines and primary cultures. By utilizing phosphatidylinositol-specific phospholipase C (PI-PLC) treatment and FR alpha-blocking antibodies, we demonstrated FR alpha-dependent and -independent entry by filovirus glycoprotein-**pseudotyped** FIV-based vectors in airway epithelia. Of particular interest, entry independent of FR alpha was observed in primary cultures of human airway epithelia. Understanding viral vector binding and entry pathways is fundamental for developing cystic fibrosis gene therapy applications.

```
=> north c l/au
NORTH IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> e north c l/au
E1            1     NORTH C I/AU
E2            2     NORTH C J/AU
E3           10 --> NORTH C L/AU
E4            3     NORTH C M/AU
E5            1     NORTH C P/AU
E6            6     NORTH C Q/AU
E7            1     NORTH C Q JR/AU
E8            1     NORTH C R/AU
E9           55     NORTH C S/AU
E10           1     NORTH C T/AU
E11           2     NORTH CAROL/AU
E12          15     NORTH CAROL S/AU

=> s e3
L15          10 "NORTH C L"/AU

=> d l15,ti,1-10

L15  ANSWER 1 OF 10     MEDLINE on STN
TI   Ross River virus glycoprotein-pseudotyped retroviruses and stable cell
     lines for their production.

L15  ANSWER 2 OF 10     MEDLINE on STN
TI   Backbone dynamics of a module pair from the ligand-binding domain of the
     LDL receptor.

L15  ANSWER 3 OF 10     MEDLINE on STN
TI   Evidence that familial hypercholesterolemia mutations of the LDL receptor
     cause limited local misfolding in an LDL-A module pair.

L15  ANSWER 4 OF 10     MEDLINE on STN
TI   Solution structure of the sixth LDL-A module of the LDL receptor.

L15  ANSWER 5 OF 10     MEDLINE on STN
TI   The folding and structural integrity of the first LIN-12 module of human
     Notch1 are calcium-dependent.

L15  ANSWER 6 OF 10     MEDLINE on STN
TI   Structural independence of ligand-binding modules five and six of the LDL
     receptor.
```

L15 ANSWER 7 OF 10 MEDLINE on STN
TI Conformational trapping in a membrane environment: a regulatory mechanism for protein activity?.

L15 ANSWER 8 OF 10 MEDLINE on STN
TI Membrane orientation of the N-terminal segment of alamethicin determined by solid-state 15N NMR.

L15 ANSWER 9 OF 10 MEDLINE on STN
TI Correlations between function and dynamics: time scale coincidence for ion translocation and molecular dynamics in the gramicidin channel backbone.

L15 ANSWER 10 OF 10 MEDLINE on STN
TI Molecular flexibility demonstrated by paramagnetic enhancements of nuclear relaxation. Application to alamethicin: a voltage-gated peptide channel.

=> d l15,cbib,ab

L15 ANSWER 1 OF 10 MEDLINE on STN
2001196568. PubMed ID: 11222688. Ross River virus glycoprotein-pseudotyped retroviruses and stable cell lines for their production. Sharkey C M; **North C L**; Kuhn R J; Sanders D A. (Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907-1392, USA.) Journal of virology, (2001 Mar) 75 (6) 2653-9. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Pseudotyped retroviruses have important applications as vectors for gene transfer and gene therapy and as tools for the study of viral glycoprotein function. Recombinant Moloney murine leukemia virus (Mo-MuLV)-based retrovirus particles efficiently incorporate the glycoproteins of the alphavirus Ross River virus (RRV) and utilize them for entry into cells. Stable cell lines that produce the RRV glycoprotein-pseudotyped retroviruses for prolonged periods of time have been constructed. The pseudotyped viruses have a broadened host range, can be concentrated to high titer, and mediate stable transduction of genes into cells. The RRV glycoprotein-pseudotyped retroviruses and the cells that produce them have been employed to demonstrate that RRV glycoprotein-mediated viral entry occurs through endocytosis and that membrane fusion requires acidic pH. Alphavirus glycoprotein-pseudotyped retroviruses have significant advantages as reagents for the study of the biochemistry and prevention of alphavirus entry and as preferred vectors for stable gene transfer and gene therapy protocols.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2

```
L9              2 S L8 NOT L6
                E KUHN R J/AU
L10            95 S E3
L11             9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12             8 S L11 NOT (L6 OR L9)
                E JEFFERS S A/AU
L13            33 S E2 OR E5
L14             2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15            10 S E3

=> file wpids
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         8.16      14.26

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
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FILE LAST UPDATED:          5 MAR 2004      <20040305/UP>
MOST RECENT DERWENT UPDATE:     200416        <200416/DW>
DERWENT WORLD PATENTS INDEX SUBSCRIBER FILE, COVERS 1963 TO DATE


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    DERWENT UPDATE 200403.
    THE TIME RANGE CODE WILL ALSO CHANGE FROM 018 TO 2004.
    SDIS USING THE TIME RANGE CODE WILL NEED TO BE UPDATED.
    FOR FURTHER DETAILS: http://thomsonderwent.com/chem/polymers/ <<<


=> e sanders d a/in
E1              3     SANDERS C W/IN
E2             22     SANDERS D/IN
E3              4 --> SANDERS D A/IN
E4             10     SANDERS D C/IN
E5              2     SANDERS D D/IN
E6             36     SANDERS D E/IN
E7             15     SANDERS D G/IN
E8             37     SANDERS D J/IN
E9              1     SANDERS D J L/IN
E10             1     SANDERS D K/IN
E11             5     SANDERS D L/IN
E12             7     SANDERS D M/IN

=> s e3
L16             4 "SANDERS D A"/IN

=> d l16,ti,1-4

L16  ANSWER 1 OF 4  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   New pseudotyped retrovirus comprising recombinant RNA associated with a
     retroviral core surrounded by a lipid bilayer of a glycoprotein comprising
     a modified O-glycosylation region, useful for transducing target cells and
```

in gene therapy.

L16 ANSWER 2 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI New pseudotyped lentivirus comprising a lentiviral capsid, a lipid bilayer and a Marburg glycoprotein disposed in the lipid bilayer, useful for eliciting an immune response against feline immunodeficiency virus infection.

L16 ANSWER 3 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Cells that produce inventive pseudotyped retroviruses having a broad host range useful for introducing nucleotide sequences into target cells.

L16 ANSWER 4 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
TI Assessing activity of substance as potassium channel agonist - to test substances for use as hair growth promoters for treatment of baldness.

=> d l16,bib,ab,1-3

L16 ANSWER 1 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2004-043127 [04] WPIDS
DNC C2004-017856
TI New pseudotyped retrovirus comprising recombinant RNA associated with a retroviral core surrounded by a lipid bilayer of a glycoprotein comprising a modified O-glycosylation region, useful for transducing target cells and in gene therapy.
DC B04 D16
IN JEFFERS, S A; SANCHEZ, A; **SANDERS, D A**
PA (CENT-N) CENTERS DISEASE CONTROL & PREVENTION; (JEFF-I) JEFFERS S A; (PURD) PURDUE RES FOUND; (SANC-I) SANCHEZ A; (SAND-I) SANDERS D A
CYC 102
PI WO 2003102219 A2 20031211 (200404)* EN 80p
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2003102219 A2 WO 2003-US17577 20030604
PRAI US 2003-458070P 20030327; US 2002-386064P 20020604
AB WO2003102219 A UPAB: 20040115
NOVELTY - A new pseudotyped retrovirus comprising recombinant RNA associated with a retroviral core surrounded by a lipid bilayer of a disposed glycoprotein comprising a modified O-glycosylation region. The recombinant RNA comprises a nucleotide sequence defining a selected biomolecule intended for delivery to a target cell, and retroviral control elements for packaging, reverse transcription and integration of the retrovirus into a target cell.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a pseudotyped retrovirus pseudotyped with a glycoprotein comprising a modified O-glycosylation region, the pseudotyped retrovirus having a transduction efficiency into a target cell of at least 2-fold higher than a retrovirus pseudotyped with the wild-type glycoproteins;

(2) a recombinant virus producer cell comprising gag, pro and pol nucleotide sequences and a nucleotide sequence encoding a glycoprotein comprising a modified O-glycosylation region;

(3) a method for making a pseudotyped retrovirus comprising supplying the recombinant RNA cited above to the recombinant virus producer cell under conditions such that pseudotyped retrovirus is produced; and

(4) a method for transducing a target cell by contacting a target cell with the pseudotyped retrovirus.

USE - The pseudotyped retroviruses are useful for transducing target cells (claimed).

ADVANTAGE - The invention provides pseudotyped retrovirus that is not toxic to cells and produces high titers of competent virus.
Dwg.0/3

L16 ANSWER 2 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2003-421416 [39] WPIDS
DNC C2003-111063
TI New pseudotyped lentivirus comprising a lentiviral capsid, a lipid bilayer and a Marburg glycoprotein disposed in the lipid bilayer, useful for eliciting an immune response against feline immunodeficiency virus infection.
DC B04 D16
IN DAVIDSON, B L; MCCRAY, P B; **SANDERS, D A**
PA (DAVI-I) DAVIDSON B L; (MCCR-I) MCCRAY P B; (SAND-I) SANDERS D A
CYC 101
PI WO 2003035849 A2 20030501 (200339)* EN 33p
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2003035849 A2 WO 2002-US34545 20021028
PRAI US 2001-356436P 20011026; US 2001-353221P 20011026
AB WO2003035849 A UPAB: 20030619
NOVELTY - A new pseudotyped lentivirus comprising:
(1) a lentiviral capsid;
(2) a lipid bilayer surrounding the capsid; and
(3) a Marburg glycoprotein (with the mutation of C671A, F67stop or Y679Astop) disposed in the lipid bilayer, is new.
DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a method of introducing a nucleic acid sequence encoding a desired protein into the airway epithelial cells of a mammal.
ACTIVITY - Virucide. No biological data given.
MECHANISM OF ACTION - Gene therapy.
USE - The lentivirus is useful for preparing a composition for eliciting an immune response against feline immunodeficiency virus infection.
Dwg.0/10

L16 ANSWER 3 OF 4 WPIDS COPYRIGHT 2004 THOMSON DERWENT on STN
Full Text
AN 2000-224030 [19] WPIDS
DNC C2000-068286
TI Cells that produce inventive pseudotyped retroviruses having a broad host range useful for introducing nucleotide sequences into target cells.
DC B04 D16
IN FISCHBACH, M A; JEFFERS, S A; KUHN, R J; NORTH, C L; **SANDERS, D A**; SHARKEY, C M
PA (PURD) PURDUE RES FOUND
CYC 87
PI WO 2000008131 A2 20000217 (200019)* EN 67p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZA ZW
AU 9954658 A 20000228 (200030)
ADT WO 2000008131 A2 WO 1999-US17702 19990804; AU 9954658 A AU 1999-54658 19990804
FDT AU 9954658 A Based on WO 2000008131
PRAI US 1998-112405P 19981215; US 1998-95242P 19980804
AB WO 200008131 A UPAB: 20000419

NOVELTY - A eukaryotic cell (C1) constructed to produce infectious retroviruses pseudotyped with viral glycoproteins where the retroviruses have a broad host range.

DETAILED DESCRIPTION - The cell (C1) comprises:

(a) a first nucleotide sequence encoding a retroviral Gag polypeptide;

(b) a second nucleotide sequence encoding a retroviral Pro polypeptide;

(c) a third nucleotide sequence encoding a retroviral Pol polypeptide; and

(d) a fourth nucleotide sequence encoding at least two different viral glycoproteins.

INDEPENDENT CLAIMS are also included for the following:

(1) a eukaryotic cell (C2) comprising (a-c) as above and (d) a fourth nucleotide sequence encoding a filoviral glycoprotein, where the first, second, third and fourth nucleotide sequences are chromosomally integrated and the cell produces pseudotyped retroviruses;

(2) a eukaryotic cell (C3) comprises (a-c) as in cells (C1) and (C2) and (d) a fourth nucleotide sequence encoding a Marburg virus glycoprotein;

(3) a pseudotyped retrovirus (R1) containing:

(a) a retroviral capsid;

(b) a lipid bilayer which surrounds the retroviral capsid; and

(c) at least two different viral glycoproteins disposed in the lipid bilayer;

(4) a method (M7) is claimed for screening agents effective in blocking viral entry into a cell by the following:

(a) treating a pseudotyped retrovirus with the agent where the pseudotyped retrovirus (R1) and a nucleotide sequence encoding a desired marker where the nucleotide sequence is enclosed within the retroviral capsid;

(b) treating a cell permissive for entry of a virus having at least two different viral glycoproteins disposed in its lipid bilayer with the treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker;

(5) a method of introducing a nucleotide sequence into a cell comprising transducing a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with a pseudotyped retrovirus having:

(a) a retroviral capsid;

(b) a lipid bilayer surrounded by the retroviral capsid;

(c) at least two different viral glycoproteins disposed in the lipid bilayer; and

(d) a desired ribonucleotide sequence;

(6) a method of introducing a nucleotide sequence into a cell comprising transducing a cell permissive for the Marburg virus entry with a pseudotyped retrovirus having:

(a) a retroviral capsid;

(b) a lipid bilayer surrounded by the retroviral capsid;

(c) at least two different viral glycoproteins disposed in the lipid bilayer; and

(d) a nucleotide sequence encoding a desired marker which is enclosed by the retroviral capsid;

(7) a method (M8) for screening agents effective in blocking Marburg virus entry into a cell by the following:

(a) treating a pseudotyped retrovirus (R2) with the agent and a nucleotide sequence encoding a desired marker where the nucleotide sequence is enclosed within the retroviral capsid;

(b) treating a cell permissive for Marburg virus entry with the treated pseudotyped retrovirus; and

(c) identifying eukaryotic cells having the desired marker;

(8) methods for screening agents effective in blocking viral entry into a cell by the following, Method (M9):

(a) treating a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with the agent;

(b) contacting the treated cell with a pseudotyped retrovirus (R1)

and a nucleotide sequence encoding a desired marker, enclosed within the retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker;

(9) a method (M10) for screening agents effective in blocking viral entry into a cells comprising:

(a) treating a cell permissive for entry of a Marburg virus with the agent;

(b) contacting the treated cell with a pseudotyped retrovirus (R2) and a nucleotide sequence encoding a desired marker, enclosed within the retroviral capsid; and

(c) identifying eukaryotic cells having the desired marker;

(10) kits for forming a pseudotyped retrovirus comprising the nucleotide sequences (a)-(d) given in cell (C1) or the nucleotide sequences (a)-(d) given in cell (C3).

USE - Methods are claimed for introducing a nucleotide sequence into a cell by transducing a cell permissive for entry of a virus having at least two different viral glycoproteins in its lipid bilayer with a pseudotyped retrovirus (R1) as in claim 7 with a desired ribonucleotide sequence (method M5). The retroviral capsid is a Moloney murine leukemia virus capsid and the virus having at least two different glycoproteins in its lipid bilayer is alpha togavirus so that the two different viral glycoproteins are alphaviral glycoproteins. A method (M6) of introducing a nucleotide sequence into a cell by transducing a cell permissive for Marburg virus entry with a pseudotyped retrovirus (R2) as in claim (8) with a desired ribonucleotide sequence is also claimed. The agents screened for effectiveness in blocking viral entry into a cell may be immunological agents such as monoclonal and/or polyclonal antibodies. The pharmacological agents include proteins, peptides or various chemical agents. The pseudotyped retrovirus may be useful in methods of identifying cell surface receptors that allow viral entry.

ADVANTAGE - Previous retroviruses used do not have a broad host range unlike the pseudotyped retroviruses provided in this invention where the envelope glycoproteins of the virus are substituted with that of a different virus. The pseudotyped retrovirus therefore has the host range of the different virus.

Dwg.0/5

```
=> e fischbach m a/in
E1            1     FISCHBACH L R/IN
E2           29     FISCHBACH M/IN
E3            1 --> FISCHBACH M A/IN
E4            1     FISCHBACH M R/IN
E5            3     FISCHBACH O/IN
E6            2     FISCHBACH P/IN
E7            1     FISCHBACH P C/IN
E8           23     FISCHBACH R/IN
E9            3     FISCHBACH R C/IN
E10          45     FISCHBACH S/IN
E11           1     FISCHBACH T/IN
E12           1     FISCHBACH T A/IN

=> s e3
L17           1 "FISCHBACH M A"/IN

=> d l17,ti

L17  ANSWER 1 OF 1  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Cells that produce inventive pseudotyped retroviruses having a broad host
     range useful for introducing nucleotide sequences into target cells.


=> e kuhn r j/in
E1            4     KUHN R F/IN
E2            3     KUHN R H/IN
E3            2 --> KUHN R J/IN
```

```
E5            3     KUHN R M/IN
E6            2     KUHN R R/IN
E7            1     KUHN R T/IN
E8           45     KUHN S/IN
E9            1     KUHN S A/IN
E10           1     KUHN S D/IN
E11           1     KUHN S E/IN
E12           1     KUHN S H/IN

=> s e3
L18           2 "KUHN R J"/IN

=> d l18,ti,1-2

L18  ANSWER 1 OF 2  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Orthodontic measurement gauge has two arms connected in such way that
     distance is maintained in between pointer tip and longitudinal extension
     with vertical extension extending towards pointer.

L18  ANSWER 2 OF 2  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Cells that produce inventive pseudotyped retroviruses having a broad host
     range useful for introducing nucleotide sequences into target cells.


=> e jeffers s a/in
E1            1     JEFFERS R P/IN
E2            2     JEFFERS S/IN
E3            3 --> JEFFERS S A/IN
E4            2     JEFFERS S E/IN
E5            1     JEFFERS S N/IN
E6            1     JEFFERS T E/IN
E7            1     JEFFERS T H/IN
E8            2     JEFFERS T K/IN
E9            3     JEFFERS W/IN
E10           1     JEFFERS W E/IN
E11           1     JEFFERS W L/IN
E12           1     JEFFERS W O/IN

=> s e3
L19           3 "JEFFERS S A"/IN

=> d l19,ti,1-3

L19  ANSWER 1 OF 3  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   New pseudotyped retrovirus comprising recombinant RNA associated with a
     retroviral core surrounded by a lipid bilayer of a glycoprotein comprising
     a modified O-glycosylation region, useful for transducing target cells and
     in gene therapy.

L19  ANSWER 2 OF 3  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Pharmaceutical composition used for forming implant in situ, comprises
     biodegradable thermoplastic polymer or copolymer, biocompatible organic
     solvent which solubilizes polymer and e.g. acetic acid compound.

L19  ANSWER 3 OF 3  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Cells that produce inventive pseudotyped retroviruses having a broad host
     range useful for introducing nucleotide sequences into target cells.


=> e north c l/in
E1            1     NORTH C J/IN
E2            1     NORTH C J G/IN
E3            1 --> NORTH C L/IN
E4            1     NORTH C P/IN
E5            1     NORTH C R/IN
```

```
E7            2      NORTH D A/IN
E8            3      NORTH D C/IN
E9            1      NORTH D D/IN
E10           1      NORTH D F/IN
E11           1      NORTH D G/IN
E12           4      NORTH D J/IN

=> s e3
L20           1 "NORTH C L"/IN

=> d l20,ti

L20  ANSWER 1 OF 1  WPIDS  COPYRIGHT 2004 THOMSON DERWENT on STN
TI   Cells that produce inventive pseudotyped retroviruses having a broad host
     range useful for introducing nucleotide sequences into target cells.


=> file medline
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      25.29      39.55

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

 FILE LAST UPDATED: 9 MAR 2004 (20040309/UP).  FILE COVERS 1953 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> file uspatful
COST IN U.S. DOLLARS                                  SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       0.38      39.93

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
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USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

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>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
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>>>  the earliest to the latest publication.                          <<<

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substance identification.

=> d his

     (FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
                E SANDERS DAVID A/IN
L1            2 S E3 OR E4
                E FISCHBACH MICHAEL A/IN
L2            1 S E4
                E KUHN RICHARD J/IN
L3            2 S E3
                E JEFFERS SCOTT A/IN
L4            1 S E3
                E NORTH CYNTHIA L/IN

     FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
                E SANDERS D A/AU
L5          245 S E2 OR E3
L6            6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
                E FISCHBACH M A/AU
L7          134 S E2
L8            2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9            2 S L8 NOT L6
                E KUHN R J/AU
L10          95 S E3
L11           9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12           8 S L11 NOT (L6 OR L9)
                E JEFFERS S A/AU
L13          33 S E2 OR E5
L14           2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15          10 S E3

     FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
                E SANDERS D A/IN
L16           4 S E3
                E FISCHBACH M A/IN
L17           1 S E3
                E KUHN R J/IN
L18           2 S E3
                E JEFFERS S A/IN
L19           3 S E3
                E NORTH C L/IN
L20           1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004

=> s (retrovir? vector? or retrovir? expression vector? or pseudotyp? particle? or pseudotype r
         29235 RETROVIR?
        182544 VECTOR?
         13196 RETROVIR? VECTOR?
                 (RETROVIR?(W)VECTOR?)
```

```
          196055 EXPRESSION
          182544 VECTOR?
            1119 RETROVIR? EXPRESSION VECTOR?
                   (RETROVIR?(W)EXPRESSION(W)VECTOR?)
             754 PSEUDOTYP?
          539686 PARTICLE?
              46 PSEUDOTYP? PARTICLE?
                   (PSEUDOTYP?(W)PARTICLE?)
             240 PSEUDOTYPE
              48 RETOVIR?
          456277 PARTICLES
                0 PSEUDOTYPE RETOVIR? PARTICLES
                   (PSEUDOTYPE(W)RETOVIR?(W)PARTICLES)
L21        13358 (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOTYP?
                  PARTICLE? OR PSEUDOTYPE RETOVIR? PARTICLES)

=> s l21 and (MoMLV or moloney murine leukemia virus)
             608 MOMLV
            6171 MOLONEY
           36873 MURINE
           27998 LEUKEMIA
           70726 VIRUS
            3921 MOLONEY MURINE LEUKEMIA VIRUS
                   (MOLONEY(W)MURINE(W)LEUKEMIA(W)VIRUS)
L22         2617 L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)

=> s l22 and (MoMLV or moloney murine leukemia virus/clm)
             608 MOMLV
             185 MOLONEY/CLM
            1873 MURINE/CLM
            3066 LEUKEMIA/CLM
           12053 VIRUS/CLM
             137 MOLONEY MURINE LEUKEMIA VIRUS/CLM
                   ((MOLONEY(W)MURINE(W)LEUKEMIA(W)VIRUS)/CLM)
L23          466 L22 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)

=> del l23
DELETE L23? (Y)/N:y

=> s l22 and (MoMLV/clm or moloney murine leukemia virus/clm)
              36 MOMLV/CLM
             185 MOLONEY/CLM
            1873 MURINE/CLM
            3066 LEUKEMIA/CLM
           12053 VIRUS/CLM
             137 MOLONEY MURINE LEUKEMIA VIRUS/CLM
                   ((MOLONEY(W)MURINE(W)LEUKEMIA(W)VIRUS)/CLM)
L23          106 L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)

=> s l23 and (gag/clm or pol/clm or pro/clm)
             973 GAG/CLM
             547 POL/CLM
            7363 PRO/CLM
L24           28 L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)

=> s l24 and ay<2000
         2989870 AY<2000
L25           21 L24 AND AY<2000

=> d l25,cbib,ab,clm,1-21

L25  ANSWER 1 OF 21  USPATFULL on STN
2003:142832 Pharmaceutical products comprising endothelial cell precursors.
    Isner, Jeffrey M., Weston, MA, United States
    Asahara, Takayuki, Arlington, MA, United States
```

(U.S. corporation)
US 6569428 B1 20030527
APPLICATION: US 1999-228020 19990111 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Pharmaceutical products are provided comprising EC progenitors for use in methods for regulating angiogenesis, i.e., for enhancing or inhibiting blood vessel formation, in a selected patient and in some preferred embodiments for targeting an angiogenesis modulator to specific locations. For example, the EC progenitors can be used to enhance angiogenesis or to deliver an angiogenesis modulator, e.g., anti- or pro-angiogenic agents, respectively to sites of pathologic or utilitarian angiogenesis. Additionally, in another embodiment, EC progenitors can be used to induce reendothelialization of an injured blood vessel, and thus reduce restenosis by indirectly inhibiting smooth muscle cell proliferation.
CLM What is claimed is:

1. A pharmaceutical product comprising a nucleic acid encoding an endothelial cell mitogen and endothelial cell (EC) progenitors, in a physiologically acceptable administrable form, wherein the EC progenitors are CD34+, Flk-1+, and tie-2+.

2. The pharmaceutical product of claim 1, wherein the nucleic acid comprises a vector.

3. The pharmaceutical product of claim 2, wherein the vector comprises sequence from a DNA or RNA virus.

4. The pharmaceutical product of claim 3, wherein the vector is a **retroviral vector**.

5. The pharmaceutical product of claim 4, wherein the **retroviral vector** comprises sequence from **moloney murine leukemia virus** or human immunodeficiency (HIV) virus.

6. The pharmaceutical product of claim 5, wherein the vector comprises human immunodeficiency virus (HIV) **gag** and **pol** genes.

7. The pharmaceutical product of claim 6, the product further comprising another vector comprising sequence from the human immunodeficiency (HIV) env gene.

8. The pharmaceutical product of claim 2, wherein the vector comprises sequence from a DNA virus.

9. The pharmaceutical product of claim 8, wherein the vector comprises sequence from at least one of pox virus, herpes virus, adenovirus, or adeno-associated virus.

10. The pharmaceutical product of claim 9, wherein the vector is replication defective.

11. The pharmaceutical product of claim 9, wherein the pox virus is orthopox or avipox.

12. The pharmaceutical product of claim 9, wherein the herpes virus is herpes simplex I virus (HSV).

13. The pharmaceutical product of claim 1, wherein the nucleic acid further comprises an operably linked promoter.

14. The pharmaceutical product of claim 13, wherein the promoter is a cytomegalovirus (CMV), Rous sarcoma virus (RSV), MMT promoter, or a native promoter.

15. The pharmaceutical product of claim 13, wherein the nucleic acid further comprises at least one enhancer.

16. The pharmaceutical product of claim 15, wherein the enhancer is a tat gene or tar element.

17. The pharmaceutical product of claim 2, wherein the vector comprises sequence encoding a selectable marker.

18. The pharmaceutical product of claim 1, wherein the encoded endothelial cell mitogen is sufficient to stimulate at least one of native EC cells to proliferate, migrate, remodel or form new sprouts from parental vessels.

19. The pharmaceutical product of claim 18, wherein the encoded endothelial cell mitogen comprises a secretory signal sequence.

20. The pharmaceutical product of claim 1, wherein the EC progenitors are angioblasts.

21. The pharmaceutical product of claim 1, wherein the EC progenitors are detectably-labeled.

22. The pharmaceutical product of 21, wherein the detectably-labeled EC progenitors are radiolabeled.

23. The pharmaceutical product of claim 1, wherein the EC progenitors are obtained from human mononuclear cells, heterologous or autologous umbilical cord blood, or peripheral blood.

24. The pharmaceutical product of claim 23, wherein the EC progenitors are obtained from the leukocyte fraction of peripheral blood.

L25 ANSWER 2 OF 21 USPATFULL on STN
2002:317300 Methods and compositions for producing viral particles.
Torrent, Christophe, Paris, FRANCE
Yeh, Patrice, Gif sur Yvette, FRANCE
Perricaudet, Michel, Ecrosnes, FRANCE
Klatzmann, David, Paris, FRANCE
Salzmann, Jean-Loup, Paris, FRANCE
Aventis Pharma S.A., Antony, FRANCE (non-U.S. corporation)Genopoietic, Paris, FRANCE (non-U.S. corporation)
US 6489142 B1 20021203
WO 9960144 19991125
APPLICATION: US 2001-700422 20010125 (9) <--
WO 1999-FR1184 19990518 <--
PRIORITY: FR 1998-6258 19980518
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention concerns methods and constructs for producing retroviral particles, in vitro, ex vivo or in vivo. It also concerns the use of said methods and constructs for transferring nucleic acids into cells. More particularly, the invention concerns a composition comprising the whole set of genetic elements required for constituting a retroviral particle, incorporated in one or several recombinant adenoviruses defective for all or part of the regions E1 and E4 at least (adenoviral/retroviral chimeric vectors).
CLM What is claimed is:
1. Composition comprising the whole set of genetic elements required for constituting a retroviral particle, incorporated in one or several recombinant adenoviruses defective for all or part of the E1 and E4 regions at least.

2. Composition according to claim 1, wherein the genetic elements comprise a **retroviral vector** and nucleic acids coding for retroviral

complementation functions.

3. Composition according to claim 2, wherein the genetic elements comprise: a nucleic acid coding for a retroviral **gag** protein, a nucleic acid coding for a retroviral **pol** protein, a nucleic acid coding for an envelope protein, and a nucleic acid comprising, between two LTR regions, a retroviral packaging sequence and a nucleic acid sequence of interest.

4. Composition according to claim 1, wherein said genetic elements are incorporated in a same recombinant adenovirus.

5. Composition according to claim 1, wherein said genetic elements are distributed into two recombinant adenoviruses.

6. Composition according to claim 5, wherein it comprises a first recombinant adenovirus comprising, incorporated in its genome, one or several nucleic acids coding for retroviral **gag** and **pol** proteins, and a second recombinant adenovirus comprising, incorporated in its genome, a nucleic acid coding for an envelope protein, and a nucleic acid comprising, between two LTR regions, a retroviral packaging sequence and a nucleic acid sequence of interest.

7. Composition according to claim 1, wherein said genetic elements are distributed into three recombinant adenoviruses.

8. Composition according to claim 3, wherein the **gag** and **pol** proteins are proteins from retroviruses chosen from among **MoMLV**, ALV, BLV, MMTV, and RSV.

9. Composition according to claim 3, wherein the envelope protein is a viral or cellular protein allowing retroviral particles to infect human cells.

10. Composition according to claim 9, wherein the envelope protein is an envelope protein from a GALV, A4070, RD114, VSV-G or rabies virus.

11. Composition according to claim 3, wherein the LTR region or regions are complete retroviral LTR regions or subdomains allowing reconstitution of complete LTRs after reverse transcription.

12. Composition according to claim 11, wherein the 5' LTR is deleted for the U3 domain and the 3' LTR is deleted for the U5 domain.

13. Composition according to claim 1 wherein the genetic elements allow the constitution of a lentivirus particle.

14. Composition according to claim 1, wherein the recombinant adenovirus or adenoviruses are defective for all or part of regions E1, E4 and E3.

15. Composition according to any claim 1, wherein the recombinant adenovirus or adenoviruses are defective for all or part of regions E1, E4 and E2.

16. Composition according to any claim 1, wherein the recombinant adenovirus or adenoviruses are defective for all or part of regions E1, E4 and E2 and E3.

17. Composition according to any claim 14, wherein the recombinant adenovirus or adenoviruses are further defective for all or part of the genes encoding the adenoviral late functions.

18. Composition according to any claim 1, wherein the recombinant adenovirus or adenoviruses are defective for any viral coding region.

19. Recombinant adenovirus defective for all or part of regions E1 and

several nucleic acids coding for retroviral **gag** and **pol** proteins.

20. Defective recombinant adenovirus, wherein it comprises, incorporated in its genome, a nucleic acid coding for an envelope protein, and a nucleic acid comprising, between two complete or not LTR regions, a retroviral packaging sequence and a nucleic acid sequence of interest.

21. Composition comprising a recombinant adenovirus according to claim 19 and a recombinant adenovirus according to claim 20.

22. Cell modified by a composition according to claim 1 or 21 for the preparation of a product intended for transferring nucleic acids into cells in vivo.

23. Method for producing retroviral particles in vitro, comprising incubating cells in the presence of a composition according to claim 1.

24. Method according to claim 23, wherein the ceils are cells of human origin.

25. A composition comprising one or several E1, E4-defective recombinant adenoviruses wherein said one or several recombinant adenoviruses comprise, alone or in combination, nucleic acid sequences encoding, upon expression, a retroviral particle expressing a retroviral envelope and comprising a genome.

26. A composition comprising one or several E1, E4-defective recombinant adenoviruses, wherein said defective recombinant adenoviruses comprise a defective genome, said defective genome comprising nucleic acid sequences of a **retroviral vector** and of retroviral structural proteins.

L25 ANSWER 3 OF 21 USPATFULL on STN
2002:152457 Gene delivery system and methods of use.
Kasahara, Noriyuki, Los Angeles, CA, United States
Logg, Christopher Reid, Studio City, CA, United States
Anderson, W. French, San Marino, CA, United States
University of Southern California, Los Angeles, CA, United States (U.S. corporation)
US 6410313 B1 20020625
APPLICATION: US 1999-409650 19991001 (9) <--
PRIORITY: US 1998-102933P 19981001 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A recombinant replication competent retrovirus for gene deliver and gene therapy is provided. The recombinant retrovirus has a heterologous nucleic acid sequence, a sequence encoding a cell- or tissue-specific ligand or a sequence for transcriptional targeting, or a combination of both a cell- or tissue-specific ligand and a cell- or tissue-specific transcriptional targeting sequence.
CLM What is claimed is:
1. A recombinant replication competent retrovirus comprising: a retroviral **GAG** protein; a retroviral **POL** protein; a retroviral envelope; an oncoretroviral polynucleotide sequence comprising Long-Terminal Repeat (LTR) sequences at the 5' and 3' end of the oncoretroviral genome, wherein a tissue-specific promoter sequence is contained within the U3 region of the LTR sequence at the 5' or 3' or 5' and 3' end of the oncoretroviral polynucleotide sequence, a heterologous nucleic acid sequence operably linked to a regulatory nucleic acid sequence; and cis-acting nucleic acid sequences involved in reverse transcription, packaging and integration in a target cell.

2. The retrovirus of claim 1, wherein the oncoretroviral polynucleotide sequence from an oncoretrovirus selected is from the group consisting of murine leukemia virus (MLV), **Moloney murine leukemia virus**

(MLV), gibbon ape leukemia virus (GALV), and human foamy virus (HFV).

3. The retrovirus of claim 2, wherein the MLV is an amphotropic MLV.

4. The retrovirus of claim 1, wherein the retroviral envelope comprises a chimeric protein.

5. The retrovirus of claim 4, wherein the chimeric protein comprises an ENV protein and a targeting polypeptide.

6. The retrovirus of claim 5, wherein the ENV protein is selected from the group consisting of a murine leukemia virus (MLV) ENV protein and vesicular stomatitis virus (VSV) ENV protein.

7. The retrovirus of claim 5, wherein the targeting polypeptide is an antibody, a receptor, or a receptor ligand.

8. The retrovirus of claim 5, wherein the ENV protein is an amphotropic protein.

9. The retrovirus of claim 5, wherein the ENV protein is a ecotropic protein.

10. The retrovirus of claim 1, wherein the target cell is a cell having a cell proliferative disorder.

11. The retrovirus of claim 1, wherein the target cell is a neoplastic cell.

12. The retrovirus of claim 10, wherein the cell proliferative disorder is selected from the group consisting of lung cancer, colon-rectum cancer, breast cancer, prostate cancer, urinary tract cancer, uterine cancer lymphoma, oral cancer, pancreatic cancer, leukemia, melanoma, stomach cancer and ovarian cancer.

13. The retrovirus of claim 1, wherein the promoter sequence is operably linked with a growth regulatory gene.

14. The retrovirus of claim 1, wherein the heterologous polynucleotide sequence is a suicide gene.

15. The retrovirus of claim 14, wherein the suicide gene encodes a thymidine kinase.

16. The retrovirus of claim 1, wherein the heterologous sequence is a marker gene.

17. The retrovirus of claim 1, wherein the regulatory nucleic acid sequence operably linked with the heterologous nucleic acid sequence is selected from the group consisting of a promoter, an enhancer, and an internal ribosome entry site.

18. A recombinant retroviral polynucleotide, comprising: a polynucleotide sequence encoding a **GAG** protein; a polynucleotide sequence encoding a **POL** protein; a polynucleotide sequence encoding a retroviral envelope; a polynucleotide sequence derived from an oncoretrovirus comprising a Long Terminal Repeat (LTR) at the 5' and 3' end of the retroviral polynucleotide sequence wherein a target-specific promoter sequence is contained within the U3 region of the LTR sequences at the 5' and 3' or 5' and 3' end of the retroviral polynucleotide; a heterologous polynucleotides sequence operably linked to a regulatory nucleic acid sequence; and cis acting polynucleotide sequence necessary for reverse transcription, packaging and integration in a target cell.

19. The polynucleotide of claim 18, wherein the **GAG**, **POL** and retroviral envelope polynucleotide sequences are derived from murine

leukemia virus (MLV) or Moloney murine leukemia virus (MoMLV).

20. The polynucleotide of claim 19, wherein the **MoMLV** is an amphotropic **MoMLV**.

21. The polynucleotide of claim 20, wherein the polynucleotide sequence encoding a retroviral envelope encodes a chimeric protein.

22. The polynucleotide of claim 21, wherein the chimeric protein comprises an ENV protein and a targeting polypeptide.

23. The polynucleotide of claim 22, wherein the ENV protein is selected from the group consisting of a murine leukemia virus (**MoMLV**) ENV protein and Vesicular stomatitis virus (VSV) ENV protein.

24. The polynucleotide of claim 22, wherein the targeting polypeptide is an antibody, a receptor, or a receptor ligand.

25. The polynucleotide of claim 23, wherein the ENV protein is an amphotropic protein.

26. The polynucleotide of claim 23, wherein the ENV protein is an ecotropic protein.

27. The polynucleotide of claim 18, wherein the target cell is a neoplastic cell.

28. The polynucleotide of claim 18, wherein the target cell has a cell proliferative disorder.

29. The polynucleotide of claim 28, wherein the cell proliferative disorder is selected from the group consisting of lung cancer, colon-rectum cancer, breast cancer, prostate cancer, urinary tract cancer, uterine cancer lymphoma, oral cancer, pancreatic cancer, leukemia, melanoma, stomach cancer, thyroid cancer, liver cancer, and brain cancer and ovarian cancer.

30. The polynucleotide of claim 18, wherein the promoter sequence is associated with a growth regulatory gene.

31. The polynucleotide of claim 18, wherein the heterologous polynucleotide sequence is a suicide gene.

32. The polynucleotide of claim 31, wherein the suicide gene encodes a thymidine kinase or a purine nucleoside phosphorylase (PNP).

33. The polynucleotide of claim 18, wherein the heterologous sequence is a marker gene.

34. The polynucleotide of claim 18, wherein the regulatory nucleic acid sequence operably linked with the heterologous nucleic acid sequence is selected from the group consisting of a promoter, an enhancer, and an internal ribosome entry site.

35. The polynucleotide of claim 18, wherein the polynucleotide sequence is contained in a viral particle.

36. The polynucleotide of claim 18, wherein the polynucleotide sequence is contained in a pharmaceutically acceptable carrier.

37. A recombinant replication competent murine leukemia virus (MLV), comprising: an MLV **GAG** protein; an MLV **POL** protein; an MLV envelope; an MLV polynucleotide sequence comprising Long-Terminal Repeat (LTR) sequences at the 5' and 3' end of the retroviral genome, wherein a target-specific promoter sequence is contained within the LTR sequences at the 5' and 3' end of the MLV polynucleotide sequence, a heterologous

nucleic acid sequence operably linked to a regulatory nucleic acid sequence; and cis-acting nucleic acid sequences necessary for reverse transcription, packaging and integration in a target cell.

38. A recombinant replication competent retrovirus comprising: a retroviral **GAG** protein; a retroviral **POL** protein; a retroviral envelope comprising a chimeric env protein comprising a targeting ligand; an oncoretroviral polynucleotide sequence comprising Long-Terminal Repeat (LTR) sequences at the 5' and 3' end of the oncoretroviral polynucleotide sequence, wherein a tissue-specific promoter sequence is contained within the U3 region of the LTR sequences at the 5' or3' or 5' and 3' end of the oncoretroviral polynucleotide sequence, a heterologous nucleic acid sequence operably linked to a regulatory nucleic acid sequence; and cis-acting nucleic acid sequences involved in reverse transcription, packaging and integration in a target cell.

39. A recombinant retroviral polynucleotide, comprising: a polynucleotide sequence encoding a **GAG** protein; a polynucleotide sequence encoding a **POL** protein; a polynucleotide sequence encoding a retroviral envelope, wherein said envelope comprises a chimeric env protein comprising a targeting ligand; a polynucleotide sequence derived from an oncoretrovirus comprising a Long Terminal Repeat (LTR) at the 5' and 3' end of the retroviral polynucleotide, wherein a tissue-specific promoter sequence is contained within the U3 region of the LTR sequences at the 5' or 3' or 5' and 3' end of the retroviral polynucleotide; a heterologous polynucleotide sequence operably linked to a regulatory nucleic acid sequence; and cis acting polynucleotide sequence necessary for reverse transcription, packaging and integration in a target cell.

L25 ANSWER 4 OF 21 USPATFULL on STN
2002:112895 RIBOZYMES TARGETING THE RETROVIRAL PACKAGING SEQUENCE EXPRESSION CONSTRUCTS AND RECOMBINANT RETROVIRUSES CONTAINING SUCH CONSTRUCTS.
SYMONDS, GEOFFREY P., ROSE BAY, AUSTRALIA
SUN, LUN-QUAN, RYDE, AUSTRALIA
US 2002058636 A1 20020516
APPLICATION: US 1995-375291 A1 19950118 (8) <--
PRIORITY: WO 1995-IB50 19950105
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention is directed to a synthetic non-naturally occurring oligonucleotide compound which comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus. Preferably, the viral packaging sequence is a retrovirus packaging sequence or the HIV-1 Psi packaging sequence. The RNA virus may be HIV-1, Feline Leukemia Virus, Feline Immunodeficiency Virus or one of the viruses listed in Table I. The conserved catalytic region may be derived from a hammerhead ribozyme, a hairpin ribozyme, a hepatitis delta ribozyme, an RNAase P ribozyme, a group I intron, a group II intron. The invention is also directed to multiple ribozymes, combinations of ribozymes, with or without antisense, and combinations of ribozymes, with antisense, and TAR decoys, polyTARs and RRE decoys targeted against the RNA virus. Vectors are also described. Further, methods of treatment and methods of use both in vivo and ex vivo are described.

CLM What is claimed is:

1. A synthetic non-naturally occurring oligonucleotide compound which comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus.

2. The compound of claim 1, wherein the viral packaging sequence of is a retrovirus packaging sequence.

3. The compound of claim 1, wherein the packaging sequence is the HIV-1 Psi packaging sequence.

4. The compound of claim 1, wherein the RNA virus is a Feline Leukemia Virus.

5. The compound of claim 1, wherein the RNA virus is a Feline Immunodeficiency Virus.

6. The compound of claim 1 having the structure: ##STR4## wherein each X represents a nucleotide which is the same or different and may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5' defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_n$, defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of the RNA virus; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; wherein a represents an integer which defines a number of nucleotides with the proviso that a may be 0 or 1 and if 0, the A located 5' of $(X)_a$ is bonded to the X located 3' of $(X)_a$; wherein each of m and m' represents an integer which is greater than or equal to 1; wherein $(X)_b$ represents an oligonucleotide and b represents an integer which is greater than or equal to 2.

7. The compound of claim 1 having the structure: ##STR5## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'--AAG . . . AGUCX--5 defines the conserved catalytic region; wherein each of $(X)_{nA}$ and $(X)_n$, defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein m represents an integer from 2 to 20; and wherein none of the nucleotides $(X)_m$ are Watson-Crick base paired to any other nucleotide within the compound.

8. The compound of claim 1 having the structure: ##STR6## wherein each X is the same or different and represents a ribonucleotide or a deoxyribonucleotide which may be modified or substituted in its sugar, phosphate or base; wherein each of A, C, U, and G represents a ribonucleotide which may be unmodified or modified or substituted in its sugar, phosphate or base; wherein 3'$(X)_{P4}$ . . . $(X)_{P1--5}$' defines the conserved catalytic region; wherein each of $(X)_{F4}$ and $(X)_{F3}$ defines the nucleotides whose sequence is capable of hybridizing with the predetermined target sequence within the packaging sequence of an RNA virus; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein F3 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F3 is greater than or equal to 3; wherein F4 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F4 is from 3 to 5; wherein each of $(X)_{P1}$ and $(X)_{P4}$ represents an oligonucleotide having a predetermined sequence

each of $(X)_{P4}$ base pairs with 3-5 bases of $(X)_{P1}$, wherein P1 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that P1 is from 3 to 6 and the sum of P1 and F4 equals 9; wherein each of $(X)_{P2}$ and $(X)_{P3}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P2}$ base-pairs with at least 3 bases of $(X)_{P3}$; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; and wherein $(X)_{L2}$ represents an oligonucleotide which may be present or absent with the proviso that L2 represents an integer which is greater than or equal to 3 if $(X)_{L2}$ is present.

9. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region are from the hepatitis delta virus conserved region.

10. The compound of claim 1, wherein the nucleotides whose sequences define a conserved catalytic region contain the sequence NCCA at its 3' terminus.

11. A synthetic non-naturally occurring oligonucleotide compound which comprises two or more domains which may be the same or different wherein each domain comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence is capable of hybridizing with a predetermined target sequence within a packaging sequence of an RNA virus.

12. The compound of claim 1 and further comprising a covalently linked antisense nucleic acid compound capable of hybridizing with a predetermined sequence, which may be the same or different, within a packaging sequence of the RNA virus.

13. The compound of claim 1, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sequence in the **MOMLV**, and site 749 in the HIV Psi packaging site.

14. A compound comprising the compound of claim 1, and further comprising at least one additional synthetic non-naturally occuring oligonucleotide compound with or without an antisense molecule covalently linked, and targeted to a different gene of the RNA virus genome.

15. The compound of claim 14, wherein the RNA virus is HIV and the different region of the HIV genome is selected from the group consisting of long terminal repeat, 5' untranslated region, splice donor-acceptor sites, primer binding sites, 3' untranslated region, **gag**, **pol**, protease, integrase, env, tat, rev, nef, vif, vpr, vpu, vpx, or tev region.

16. The compound of claim 15, wherein the nucleotides are capable of hybridizing with the 243, 274, 366 or 553 target sites or combination thereof in the **MOMLV** and site 749 in the HIV Psi packaging site and the nucleotides of the additional compound are capable of hybridizing with the 5792, 5849, 5886, or 6042 target sites or combination thereof in the HIV tat region.

17. A composition which comprises the compound of claims 1 or 14 in association with a pharmaceutically, veterinarially, or agriculturally acceptable carrier or excipient.

18. A composition which comprises the compound of claim 1, with or

wherein said antisense, and further comprises a HIV decoy, polyHIV or a HIV decoy.

19. A method for producing the compound of claim 1 which comprises the steps of: (a) ligating into a transfer vector comprised of DNA, RNA or a combination thereof a nucleotide sequence corresponding to the compound; (b) transcribing the nucleotide sequence of step (a) with an RNA polymerase; and (c) recovering the compound.

20. A transfer vector comprised of RNA or DNA or a combination thereof containing a nucleotide sequence which on transcription gives rise to the compound of claim 1.

21. The transfer vector of claim 20, wherein the transfer vector comprises the HIV long terminal repeat, an adenovirus associated transfer vector, an SV40 promoter, Mo-MLV, or an amphotropic **retrovirus vector**.

22. The transfer vector of claim 20 further comprising a sequence directing the oligonucleotide compound to a particular organ or cell in vivo or a particular region within the cell.

23. A composition which comprises the transfer vector of claim 20 in association with a pharmaceutically, veterinarially or agriculturally acceptable carrier or excipient.

24. A prokaryotic or eukaryotic cell comprising a nucleotide sequence which is, or on transcription give s rise to the compound of claim 1.

25. The cell of claim 24, wherein the cell is a eukaryotic cell.

26. The eukaryotic cell of claim 25, wherein the cell is an animal cell.

27. The eukaryotic cell of claim 25, wherein the cell is a hematopoietic stem cell which gives rise to progenitor cells, more mature, and fully mature cells of all the hematopoietic cell lineages.

28. The eukaryotic cell of claim 25, wherein the cell is a progenitor cell which gives rise mature cells of all the hematopoietic cell lineages.

29. The eukaryotic cell of claim 25, wherein the cell is a committed progenitor cell which gives rise to a specific hematopoietic lineage.

30. The eukaryotic cell of claim 25, wherein the cell is a T lymphocyte progenitor cell.

31. The eukaryotic cell of claim 25, wherein the cell is an immature T lymphocyte.

32. The eukaryotic cell of claim 25, wherein the cell is a mature T lymphocyte.

33. The eukaryotic cell of claim 25, wherein the cell is a myeloid progenitor cell.

34. The eukaryotic cell of claim 25, wherein the cell is a monocyte/macrophage cell.

35. The use of the compound of claims 1 to protect hematopoietic stem cells, progenitor cells, committed progenitor cells, T lymphocyte progenitor cells, immature T lymphocytes, mature T lymphocytes, myeloid progenitor cells, or monocyte/macrophage cells.

36. A method to suppress HIV in an AIDS patient which comprises the introduction of the transfer vector of claim 20 into hematopoietic cells

thereby rendering the cells resistant to HIV as well as thereby suppressing HIV in an AIDS patient.

37. The method of claim 36, wherein the introduction is ex vivo and the cells are autologous or heterologous cells.

38. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted without myeloablation.

39. The method of claim 36, wherein the introduction is ex vivo and the cells are transplanted with myeloablation.

40. The method of claim 37, wherein the cells are also treated with an additional agent to inhibit or eliminate HIV-1 replication.

41. The method of claim 40, wherein the additional agent is a neutralizing antibody such as IgG1b12; a nucleoside analogues such as zidovudine (AZT), ddI, ddC, d4t; a non-nucleoside reverse transcriptase inhibitors such as nevirapine, delavirdine, lamivudine (3-TC), loviride; or a protease inhibitors such as saquinavir.

42. A method for protecting an individual from HIV infection which comprises incorporation of the transfer vector of claim 20 into the individual's cells thereby protecting that individual from the effects of high levels of the virus.

L25 ANSWER 5 OF 21 USPATFULL on STN
2001:208684 Cap-independent multicistronic **retroviral vectors**.
Adam, Mohammed A., Kirkland, Canada
Miller, A. Dusty, Seattle, WA, United States
Osborne, William Reginald Alfred, Seattle, WA, United States
Fred Hutchinson Cancer Research Center Board Regents of the University of Washington, Seattle, WA, United States (U.S. corporation)
US 6319707 B1 20011120
APPLICATION: US 1993-9338 19930126 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB **Retroviral vectors** for producing coordinately expressed polycistronic mRNA in transfected host cells. A representative retroviral construct capable of forming a proviral genome in a host cell contains a first nucleotide coding sequence, a second nucleotide coding sequence, and a third nucleotide sequence capable of hybridizing under stringent conditions to a 5' nontranslated region (NTR) of a picornavirus RNA or its complementary RNA strand. The first, second, and third nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule containing transcripts of the first, second, and third nucleotide sequences. The transcript of the third nucleotide sequence in the messenger RNA molecule contains a nucleic acid capable of forming a regulatory stem-loop nucleic acid structure followed by at least one operable AUG start codon. The regulatory stem-loop nucleic acid structure is capable of operably binding a translation initiation complex in a host cell such that the transcripts of the first and second nucleotide sequences in the messenger RNA molecule are coordinately expressed in the host cell.
CLM What is claimed is:
1. A retroviral construct capable of forming a proviral genome in a host cell, said retroviral construct comprising: a first nucleotide sequence comprising a **MoMLV** LTR, a second nucleotide sequence comprising a first coding region, a third nucleotide sequence, comprising a picornavirus NTR, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a fourth nucleotide sequence comprising a second coding region, and a fifth nucleotide sequence comprising a poly-A tail, wherein said nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule comprising transcripts of the second, third,

2. The retroviral construct of claim 1, wherein one of said first and second coding regions encodes a detectable marker.

3. The retroviral construct of claim 2, wherein the other of said first and second coding regions encodes a therapeutic gene product.

4. The retroviral construct of claim 1, comprising **MoMLV** retroviral elements for packaging and encapsidation of the retroviral RNA into a **retroviral vector** particle.

5. The retroviral construct of claim 1, wherein the transcript of the third nucleotide sequence in said messenger RNA molecule comprises a nucleic acid capable of forming a regulatory stem-loop nucleic acid structure followed by at least one operable AUG start codon.

6. The retroviral construct of claim 5, wherein the regulatory stem-loop nucleic acid structure is capable of operably binding a translation initiation complex in a host cell such that the transcripts of the second and fourth nucleotide sequences in said messenger RNA molecule are coordinately expressed in the host cell.

7. A packaging host cell transformed with the retroviral construct of claim 1, capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said proviral genome.

8. An infective **retroviral vector** particle encapsidated by the transformed packaging host cell of claim 7.

9. A producer host cell transduced with the **retroviral vector** particle of claim 8, the genome of said producer host cell comprising a first proviral genome corresponding to said retroviral construct in combination with a second proviral genome comprising a **gag** and **pol** gene and a third proviral genome comprising an env gene, said producer host cell being capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said first proviral genome.

10. An infective **retroviral vector** particle produced by the producer host cell of claim 9.

11. A target host cell transduced with the **retroviral vector** particle of claim 10, said target host cell containing said first proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

12. A producer host cell transformed with the retroviral construct of claim 1, the genome of said producer host cell comprising a first proviral genome corresponding to said retroviral construct in combination with a second proviral genome comprising a **gag** and **pol** gene and a third proviral genome comprising an env gene, said producer host cell being capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said first proviral genome.

13. An infective **retroviral vector** particle produced by the producer host cell of claim 12.

14. A target host cell transduced with the **retroviral vector** particle of claim 13, said target host cell containing said first proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

15. A target host cell transformed with the retroviral construct of

claim 1, said target host cell containing said proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

16. A retroviral construct capable of forming a proviral genome in a host cell said retroviral construct comprising: a first nucleotide sequence comprising a **MoMLV** LTR a second nucleotide sequence comprising a first coding region, a third nucleotide sequence comprising a picornavirus NTR, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a fourth nucleotide sequence comprising a second coding region, a fifth nucleotide sequence comprising a picornavirus NTR, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a sixth nucleotide sequence comprising a third coding region, and a seventh nucleotide sequence comprising a poly-A tail, wherein said nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule comprising transcripts of the said second, third, fourth, fifth, sixth, and seventh nucleotide sequences.

L25 ANSWER 6 OF 21 USPATFULL on STN
2001:178884 Construction of retroviral producer cells from adenoviral and **retroviral vectors**.
Lin, Xinli, Edmond, OK, United States
Tang, Jordan J. N., Edmond, OK, United States
Oklahoma Medical Research Foundation, Oklahoma City, OK, United States (U.S. corporation)
US 6303380 B1 20011016
APPLICATION: US 1999-301846 19990429 (9) <--
PRIORITY: US 1998-83511P 19980429 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A combination of adenoviral and **retroviral vectors** used to construct second generation packaging cells that deliver marker genes to target cells is described. A vector based upon **Moloney murine leukemia virus** (MLV) was used to deliver marker genes, and an adenovirus-based delivery system was used to deliver MLV structural genes (gagpol and env) to cultured cells. The procedure transformed the cells into new retroviral producer cells, which generate replication-incompetent retroviral particles in the culture supernatant for transferring marker genes to target cells. The titer of the retroviral-containing supernatant generated from the second generation producer cells reached above 105 cfu/ml which is comparable to the MLV-based producer cell lines currently used in human gene therapy trials. The vector and procedures are adaptable for experimental human gene therapy in which the new producer cells are transplanted into patients for continuous gene transfer.

CLM What is claimed is:
1. A method of making a producer cell that delivers a marker gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell, comprising the steps: using a **retroviral vector** to deliver a marker or therapeutic gene to the human primary cell; and using a single adenoviral-based vector to deliver the **gag**, **pol** and env structural genes to the human primary cell, thereby making a producer cell.

2. The method of claim 1, wherein the **retroviral vector** is a **Moloney murine leukemia virus** (MLV) based vector to deliver a marker or therapeutic gene to the human primary cell.

3. The method of claim 1, wherein the **retroviral vector** is a lentivirus.

4. The method of claim 1 wherein the **gag**, **pol** and env structural genes are from **Moloney murine leukemia virus**.

5. The method of claim 3 wherein the lentivirus is human

immunodeficiency virus.

6. The method of claim 3, wherein the structural **gag**, **pol** and env structural genes are from human immunodeficiency virus-1.

7. A producer cell that delivers a marker gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell transfected with a first **retroviral vector** delivering a marker or therapeutic gene to the human primary cell; and a second single adenoviral-based vector delivering the **gag**, **pol** and env structural genes to the human primary cell.

8. A system for making a producer cell that delivers a marker gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell, the system comprising: a first **retroviral vector** for delivering a marker or therapeutic gene to the human primary cell; and a second adenoviral-based vector for delivering the **gag**, **pol** and env structural genes to the human primary cell.

9. The system of claim 8 wherein the first **retroviral vector** is a **Moloney murine leukemia virus** (MLV) based vector to deliver a marker or therapeutic gene to the human primary cell.

10. The system of claim 8, wherein the first **retroviral vector** is a lentivirus.

11. The system of claim 8 wherein the **gag**, **pol** and env structural genes are from **Moloney murine leukemia virus**.

12. The system of claim 10 wherein the lentivirus is human immodeficiency virus.

L25 ANSWER 7 OF 21 USPATFULL on STN
2001:55763 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Dull, Thomas J., San Francisco, CA, United States
Zsebo, Krisztina M., Woodside, CA, United States
Cooke, Keegan, Palo Alto, CA, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6218187 B1 20010417
APPLICATION: US 1999-266596 19990311 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.
CLM What is claimed is:
1. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a

replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

2. The method of claim 1, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

3. The method of claim 1, wherein said foreign gene encodes a chimeric T cell receptor.

4. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of; A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

5. The method of claim 4, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

6. The method of claim 4, wherein said foreign gene encodes a chimeric T cell receptor.

7. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding **gag** and **pol** proteins and a selectable marker wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

8. The method of claim 7, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

9. The method of claim 7, wherein said foreign gene encodes a chimeric T cell receptor.

10. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient transfection of a first population of mammalian cells stably transfected with at least one expression vector encoding **gag**, **pol** and env proteins and a selectable marker wherein the expression of **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

11. The method of claim 10, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

12. The method of claim 10, wherein said foreign gene encodes a chimeric T cell receptor.

13. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding adhesion molecules or antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian hematopoietic stem

cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

14. The method of claim 13, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

15. The method of claim 13, wherein said foreign gene encodes a chimeric T cell receptor.

16. The method of any one of claims 1, 4, 7, 10 or 13 wherein said adhesion molecules are selected from the group consisting of fibronectin and CS-1.

17. The method of any one of claims 1, 4, 7, 10 or 13 wherein said antibodies to adhesion molecules are selected from the group consisting of antibodies to VLA-4, VLA-5, CD29, CD11a, CD11b and CD44.

18. A method to transduce mammalian T and B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

19. The method of claim 18, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

20. The method of claim 18, wherein said foreign gene encodes a chimeric T cell receptor.

21. The method of claim 18, further comprising infecting a second population of mammalian target cells with the supernatant from said mammalian cells of claim 18 to transduce said target cells with a foreign gene.

22. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all

virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging the helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding the **gag** and **pol** proteins of ectropic MMLV or GALV and a second retroviral helper sequence comprises a cDNA encoding the envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

23. The method of claim 22, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

24. The method of claim 22, wherein said foreign gene encodes a chimeric T cell receptor.

25. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding the **gag** and **pol** proteins and a selectable marker wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

26. The method of claim 25, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

27. The method of claim 25, wherein said foreign gene encodes a chimeric T cell receptor.

28. A method to transduce mammalian T or B lymphocytes with **retroviral**

vectors produced by transient transfection comprising the steps of: A) transient transfection of a first population of mammalian cells stably transfected with at least one expression vector encoding the **gag**, **pol** and env proteins and a selectable marker wherein the expression of the **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

29. The method of claim 28, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

30. The method of claim 28, wherein said foreign gene encodes a chimeric T cell receptor.

31. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian T or B lymphocytes on said culture plates; and D) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

32. The method of claim 31, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

33. The method of claim 31, wherein said foreign gene encodes a chimeric T cell receptor.

34. The method of any one of claims 18, 22, 25, 28 or 31 wherein said antibodies to adhesion molecules is selected from the group consisting of antibodies to LFA-1, CD-2, CD40 and gp39.

35. The method of claims 1, 4, 7, 10, 13, 18, 22, 25, 28 or 31, wherein the first population of mammalian cells comprises a human cell.

36. An improved method to efficiently transduce mammalian cells with a retroviral supernatant, comprising the steps of: i) growing said population of mammalian cells on culture plates; and ii) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying a foreign gene with said population of mammalian cells, to transduce said population of mammalian cells with said foreign gene, whereby target cells efficiently transduced with said foreign gene are obtained, wherein the improvement comprises adding antibodies to adhesion molecules present on said population of mammalian cells to culture plates.

37. The target cell of claim 36, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

chimeric T cell receptor.

39. The target cell of claim 38, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

40. The method of claim 3, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

41. The method of claim 6, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

42. The method of claim 9, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

43. The method of claim 12, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

44. The method of claim 15, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

45. The method of claim 20, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

46. The method of claim 24, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

47. The method of claim 27, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

48. The method of claim 30, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

49. The method of claim 33, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

50. The method of claim 35, wherein said human cell is a 293 cell.

51. The method of claim 21, wherein said target cells are lymphocytes or hematopoietic stem cells.

L25 ANSWER 8 OF 21 USPATFULL on STN
2000:142165 Mus dunni endogenous retroviral packaging cell lines.
Miller, A. Dusty, Seattle, WA, United States
Wolgamot, Greg, Seattle, WA, United States
Bonham, Lynn, Seattle, WA, United States
Fred Hutchinson Cancer Research Center, Seattle, WA, United States (U.S. corporation)
US 6136598 20001024
APPLICATION: US 1998-75272 19980508 (9) <--
PRIORITY: US 1997-46140P 19970509 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Retroviral packaging cells produce replication-defective retroviral particles capable of binding to Mus dunni endogenous virus retroviral receptors on target cells and are useful in gene transfer and gene therapy. The packaging cell employs a vector encoding a M. dunni retroviral Env protein and produces the retroviral particles at high titer.
CLM What is claimed is:
1. A cultured packaging cell for producing a replication-defective **retroviral vector** particle which binds to Mus dunni endogenous virus receptors, wherein the packaging cell is a vertebrate cell which can express and assemble retroviral proteins, comprising: a first vector encoding a retroviral envelope protein having the amino acid sequence of a Mus dunni endogenous virus envelope protein, or a fragment thereof, that directs the binding of the **retroviral vector** particle to Mus dunni endogenous virus retroviral receptors on a target cell; and a second vector encoding retrovirus **Gag** and **Pol** proteins, wherein upon expression of said retroviral envelope, **Gag** and **Pol** proteins in the packaging cell in the presence of a third vector having a sequence of a heterologous gene of interest, produces the replication-defective **retroviral vector** particle that binds to Mus dunni endogenous virus receptors of the target cell.

2. The cultured packaging cell of claim 1, wherein the first vector

comprises an oligonucleotide sequence which encodes the amino acid sequence as depicted in SEQ ID NO:5.

3. The cultured packaging cell of claim 2, wherein the first vector comprises a nucleotide sequence as depicted in SEQ ID NO:1.

4. The cultured packaging cell of claim 2, wherein the Mus dunni endogenous virus envelope protein is a chimeric protein having non-Mus dunni envelope protein amino acid residues from a different retrovirus.

5. The cultured packaging cell of claim 4, wherein the retrovirus **Gag** and **Pol** proteins are from a **Moloney murine leukemia virus.**

6. A cultured packaging cell for producing a replication-defective **retroviral vector** particle which binds to Mus dunni endogenous virus receptors, wherein the packaging cell is a vertebrate cell which can express and assemble retroviral proteins, comprising: a first vector encoding a retroviral envelope protein having the amino acid sequence of a Mus dunni endogenous virus envelope protein, or a fragment thereof, that directs the binding of the **retroviral vector** particle to Mus dunni endogenous virus retroviral receptors on a target cell; and a second vector encoding retrovirus **Gag** and **Pol** proteins; and a third vector comprising a nucleic acid sequence encoding a heterologous gene of interest, wherein upon expression of said retroviral envelope, **Gag** and **Pol** proteins in the packaging cell produces the replication-defective **retroviral vector** particle that binds to Mus dunni endogenous virus receptors of the target cell.

7. The cultured packaging cell of claim 6, wherein the heterologous gene encodes a protein, peptide, or RNA molecule.

8. The cultured packaging cell of claim 7, wherein the RNA molecule is an antisense RNA or a ribozyme.

9. The cultured packaging cell of claim 1, wherein the vertebrate cell is an avian or mammalian cell which can express and assemble retroviral proteins.

10. The cultured packaging cell of claim 1, wherein the first vector and the second vector are integrated in a chromosome of the packaging cell.

11. The cultured packaging cell line PD223 as deposited with the American Type Culture Collection and designated CRL-12525.

12. A cultured packaging cell for producing a replication-defective **retroviral vector** particle, wherein the packaging cell is a vertebrate cell which can express and assemble retroviral proteins, comprising: a first vector comprising a Mus dunni endogenous virus envelope gene encoding the amino acid residue sequence as depicted in SEQ ID NO:5, or a fragment thereof, that directs binding of the **retroviral vector** particle to Mus dunni endogenous virus receptors on a target cell; a second vector encoding retrovirus **Gag** and **Pol** proteins; and a third vector having a sequence comprising a heterologous gene of interest, wherein upon expression of said envelope gene and said **Gag** and **Pol** proteins in the packaging cell a replication-defective **retroviral vector** particle is produced that binds to Mus dunni endogenous virus receptors of the target cell.

L25 ANSWER 9 OF 21 USPATFULL on STN
2000:117548 Ribozymes targeting the **MoMLV** PSI packaging sequence and the HIV tat sequence.
Symonds, Geoffrey P., Rose Bay, Australia
Sun, Lun-Quan, Ryde, Australia
Gene Shears Pty., Ltd., New South Wales, Australia (non-U.S. corporation)
US 6114167 20000905

DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A cell comprising a synthetic non-naturally occurring oligonucleotide compound comprises nucleotides whose sequence defines a conserved catalytic region and nucleotides whose sequence hybridizes with a predetermined target sequence within a **MoMLV** Psi packaging sequence on the HIV tat sequence. The catalytic region may be derived from a hammerhead ribozyme, a hairpin ribozyme a hepatitis delta ribozyme, an PNAase P ribozyme, a group I intron or a group II intron.
CLM What is claimed is:
1. A cell comprising a viral vector producing a non-naturally occurring RNA compound which comprises nucleotides whose sequence defines a catalytic region and nucleotides whose sequence comprises sequences complementary to both a first nucleotide sequence directly 3' and a second nucleotide sequence 5' of a target site in a target molecule of a **MoMLV** or a HIV RNA virus, wherein the target site comprises three nucleotides of which the 5' nucleotide is selected form the group consisting of nucleotide numbers 274 and 366 of the **MoMLV** packaging sequence, and nucleotide number 5849 of the HIV tat gene, wherein the non-naturally occurring RNA compound inactivates the target molecule in the cell.

2. The cell of claim 1, wherein the compound has the structure (SEQ ID NO:1): ##STR4## wherein each X represents a ribonucleotide which is the same or different; wherein each of A, C, U, and G represents a ribonucleotide; wherein 3'--AAG . . . AGUCX--5' defines the catalytic region; wherein $(X)_n$ A defines nucleotides whose sequence comprises a sequence which hybridizes to a nucleotide sequence directly 5' of the 3' nucleotide of the target site within the **MoMLV** packaging sequence or the HIV tat gene, and n defines the number of nucleotides; wherein $(X)_{n'}$ defines nucleotides whose sequence comprises a sequence which hybridizes to a nucleotide sequence directly 3' of the target site within the **MoMLV** packaging sequence or the HIV tat gene, and n' defines the number of nucleotides; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a covalent bond between the nucleotides located on either side thereof; wherein a represents an integer which defines a number of nucleotides with the proviso that a may be 0 or 1 and if 0, the A located 5' of $(X)_a$ is bonded to the X located 3' of $(X)_a$; wherein each of m and m' represents an integer which is greater than or equal to 1; and wherein $(X)_b$ represents an oligonucleotide and b represents an integer which is greater than or equal to 2.

3. The cell of claim 1, wherein the compound has the structure (SEQ ID NO:2): ##STR5## wherein each X is the same or different and represents a ribonucleotide; wherein each of A, C, U, and G represents a ribonucleotide; wherein 3'--AAG . . . AGUCX--5' defines the catalytic region; wherein $(X)_n$ A defines nucleotides whose sequence comprises a sequence which hybridizes to a nucleotide sequence directly 5' of the 3' nucleotide of the target site within the **MoMLV** packaging sequence or the HIV tat gene, and n defines the number of nucleotides; wherein $(X)_{n'}$ defines nucleotides whose sequence comprises a sequence which hybridizes to a nucleotide sequence directly 3' of the target site within the **MoMLV** packaging sequence or the HIV tat gene, and n defines the number of nucleotides; wherein each solid line represents a covalent bond between the nucleotides located on either side thereof; and wherein m represents an integer from 2 to 20; and wherein none of the nucleotides $(X)_m$ are Watson-Crick base paired to any other nucleotide within the compound.

4. The cell of claim 1, wherein the compound has the structure (SEQ ID NO:3): ##STR6## wherein each X is the same or different and represents a ribonucleotide; wherein each of A, C, U, and G represents a

nucleotide, wherein $(X)_{P4}$ . . . $(X)_{P1}$ defines the catalytic region; wherein $(X)_{F4}$ defines the nucleotides whose sequence comprises a sequence complementary to a nucleotide sequence 5' of the target site in the target molecule of the **MoMLV** or HIV RNA virus; wherein $(X)_{F3}$ defines the nucleotides whose sequence comprises a sequence complementary to a nucleotide sequence directly 3' of the target site in the target molecule of the **MoMLV** or HIV RNA virus; wherein F3 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F3 is greater than or equal to 3; wherein F4 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that F4 is from 3 to 5; wherein each of $(X)_{P1}$ and $(X)_{P4}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P4}$ base-pairs with 3-6 bases of $(X)_{P1}$; wherein P1 represents an integer which defines the number of nucleotides in the oligonucleotide with the proviso that P1 is from 3 to 6 and the sum of P1 and F4 equals 9; wherein each of $(X)_{P2}$ and $(X)_{P3}$ represents an oligonucleotide having a predetermined sequence such that $(X)_{P2}$ base-pairs with at least 3 bases of $(X)_{P3}$; wherein each * represents base pairing between the nucleotides located on either side thereof; wherein each solid line represents a chemical linkage providing covalent bonds between the nucleotides located on either side thereof; wherein each of the dashed lines independently represents either a chemical linkage providing covalent bonds between the nucleotides located on either side thereof or the absence of any such chemical linkage; and wherein $(X)_{L2}$ represents an oligonucleotide which may be present or absent with the proviso that L2 represents an integer which is greater than or equal to 3 if $(X)_{L2}$ is present.

5. The cell of claim 1, wherein the nucleotides whose sequence defines a catalytic region are from a hepatitis delta virus conserved region.

6. The cell of claim 1, wherein the catalytic region contains the sequence NCCA at its 3' terminus.

7. The cell of claim 1, wherein the compound is covalently linked to an antisense nucleic acid compound that hybridizes to a predetermined sequence of the **MoMLV** or HIV virus located 3' or 5' of the target site.

8. The cell of claim 1, wherein the cell is a human cell and the virus is HIV, and wherein the compound further comprises at least one additional catalytic region, with or without an antisense molecule covalently linked thereto, and targeted to a different region of the genome of the HIV virus.

9. The cell of claim 8, wherein the different region of the HIV genome is selected from the group consisting of long terminal repeat, 5' untranslated region, splice donor-acceptor sites, primer binding sites, 3' untranslated region, **gag**, **pol**, protease, integrase, env, tat, rev, nef, vif, vpr, vpu, vpx, and tev region.

10. The cell of claim 9, wherein each of the additional catalytic regions is linked to nucleotide sequences complementary to both a first nucleotide sequence directly 3' and a second nucleotide sequence 5' of a target site wherein the 5' nucleotide of the target site is selected from the group consisting of nucleotide numbers 5792, 5849, 5886, and 6042 in the HIV tat region and nucleotide number 749 in the HIV packaging sequence.

11. A cell comprising a viral vector which produces a non-naturally occurring RNA compound which comprises two or more domains which may be the same or different wherein at least one domain comprises nucleotides whose sequence defines a catalytic region and nucleotides whose sequence comprises sequences complementary to both a first nucleotide sequence

target molecule of a **MoMLV** or a HIV RNA virus, wherein the target site consists of three nucleotides of which the 5' nucleotide is selected form the group consisting of nucleotide numbers 274 and 366 of the **MoMLV** packaging sequence, and nucleotide number 5849 of the HIV tat gene, wherein the non-naturally occurring RNA compound inactivates the target molecule in the cell.

12. A human cell resistant to HIV infection comprising a viral vector producing a non-naturally occurring RNA compound which comprises nucleotides whose sequence defines a catalytic region that catalyzes RNA cleavage and nucleotides whose sequence defines a first nucleotide sequence that hybridizes to a first portion of SEQ ID NO. 10 and a second nucleotide sequence that hybridizes to a second portion of SEQ ID NO. 10, wherein the non-naturally occurring RNA compound inactivates an HIV tat gene comprising SEQ ID NO. 10 in the human cell, thus making the human cell resistant to HIV infection.

L25 ANSWER 10 OF 21 USPATFULL on STN
2000:47084 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Roberts, Margo R., Charlottesville, VA, United States
Dull, Thomas L., San Francisco, CA, United States
Zsebo, Krisztina M., Cupertino, CA, United States
Qin, Lu, Lutherville, MD, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6051427 20000418
APPLICATION: US 1995-517488 19950821 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.
CLM What is claimed is:
1. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-imcompetent retroviral genome encoding in trans all virion proteins required and for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said helper DNA sequence codes for ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV) **gag** and **pol**, and an envelope protein or chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia

leukemia virus (HTLV) type I and HTLV type II.

2. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV), and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

3. The stable packaging cell line comprising helper sequences encoding **gag** and **pol** proteins designated 35.32.

4. A human embryonic kidney cell stably transfected with an expression vector encoding **gag** and **pol** proteins and a selectable marker, wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent.

5. The human embryonic kidney cell of claim 4 wherein the **gag** and **pol** are derived from **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV).

6. The human embryonic kidney cell of claim 4 wherein said cell is either 293 or tsa54.

7. A human embryonic kidney cell stably transfected with two expression vectors wherein the first expression vector encodes **gag** and **pol** proteins and the second expression vector encodes an envelope protein.

8. The human embryonic kidney cell of claim 7 wherein said cell is either 293 or tsa54 and said gal and **pol** proteins and derived from **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV).

9. The human embryonic kidney cell of claim 7 or 10 wherein said envelope protein is derived from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

10. The human embryonic kidney cell of claim 9, wherein said envelope protein is comprised of sequences from two or more of said viruses.

11. A stable packaging cell line comprising helper sequences encoding **gag, pol** and envelope proteins designated 37S2.8.

12. A retroviral packaging plasmid for the production of high titers of

recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking the both the psi function sequence responsible for packaging helper genome and the 3'LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said foreign enhancer is the RSV enhancer and promoter.

13. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or immunodeficiency virus (HIV) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II; and wherein said foreign enhancer is the RSV enhancer and promoter.

L25 ANSWER 11 OF 21 USPATFULL on STN
2000:27801 Gibbon ape leukemia virus-based **retroviral vectors**.
Eiden, Maribeth V., Bethesda, MD, United States
Wilson, Carolyn A., Arlington, VA, United States
Deacon, Nicholas J., Balwyn, Australia
Hooker, David J., Mill Park, Australia
The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
US 6033905 20000307
WO 9423048 19941013
APPLICATION: US 1997-716351 19970224 (8) <--
WO 1994-US3784 19940406 19970224 PCT 371 date 19970224 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides replication-defective hybrid **retroviral vectors** comprising GaLV components and methods for preparing and using such vectors. The vectors comprise a envelope component, a core component and a defective genome, at least one of which is derived from GaLV. The vectors can comprise the minimal cis acting sequences from GaLV that allow packaging of the defective genome in a hybrid virion.
CLM What is claimed is:
1. A recombinant DNA construct comprising a replication-defective retroviral genome comprising a polynucleotide sequence of interest and a gibbon ape leukemia virus (GaLV) packaging site.

2. The construct of claim 1, wherein the packaging site consists of

between about 150 base pairs and about 1500 base pairs.

3. The construct of claim 1, wherein the packaging site consists of a sequence extending from about position 570 to about position 1280 of SEQ ID NO:1.

4. The construct of claim 1, wherein the construct further comprises regulatory sequences which direct expression of the polynucleotide of interest.

5. The construct of claim 4, wherein the regulatory sequences are from a GaLV 3' LTR.

6. The construct of claim 5, wherein the regulatory sequences are from GaLV SF.

7. The construct of claim 1, wherein the construct comprises a sequence encoding the GaLV envelope (env) glycoprotein.

8. A cultured mammalian cell comprising the replication-defective viral genome of claim 1.

9. The cell of claim 8, further comprising retroviral **gag** and **pol** genes.

10. The cell of claim 9, wherein the **gag** and **pol** genes are from GaLV SF or GaLV SEATO.

11. The cell of claim 9, wherein the **gag** and **pol** genes are from **MoMLV**.

12. The cell of claim 8, further comprising a retroviral env gene.

13. The cell of claim 12, wherein the env gene is from GaLV SF or GaLV SEATO.

14. The cell of claim 8, which is PG13 or PA317.

15. An isolated hybrid retrovirus virion comprising, a GaLV envelope protein, an RNA genome comprising a polynucleotide sequence of interest operably linked to expression regulatory sequences, and, a GaLV packaging site.

16. The virion of claim 15, further comprising GaLV core proteins.

17. The virion of claim 15, further comprising **MoMLV** core proteins.

18. The virion of claim 15, wherein the envelope protein is a GaLV SF envelope protein.

19. The virion of claim 15, wherein the packaging site is transcribed from a sequence consisting of between about 150 base pairs and about 1500 base pairs.

20. The virion of claim 15, wherein the packaging site is transcribed from a polynucleotide sequence extending from about position 570 to about position 1280 of SEQ ID NO:1.

21. A method of introducing a polynucleotide sequence of interest into human cells having a GaLV receptor, comprising: contacting the cells, in vitro, with hybrid retrovirus virions comprising, a GaLV envelope protein, an RNA genome comprising the polynucleotide sequence of interest operably linked to expression regulatory sequences, and a GaLV packaging site, selecting cells having the polynucleotide of interest.

22. The method of claim 21, wherein the human calls are selected from the group consisting of bone marrow cells and tumor infiltrating cells.

L25 ANSWER 12 OF 21 USPATFULL on STN
2000:9750 Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant.
Rigg, Richard J., Mountain View, CA, United States
Chen, Jingyi, Fremont, CA, United States
Dando, Jonathan S., Milan, Italy
Plavec, Ivan, Menlo Park, CA, United States
Forestell, Sean P., Menlo Park, CA, United States
Bohnlein, Ernst, Los Altos, CA, United States
SyStemix, Inc., Palo Alto, CA, United States (U.S. corporation)
US 6017761 20000125
WO 9721825 19970619
APPLICATION: US 1997-817452 19970415 (8) <--
WO 1996-US20777 19961213 19970415 PCT 371 date 19970415 PCT 102(e) date <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** as well as the recombinant packaging cell obtained by the method. Also provided is a method of producing recombinant retroviral particles obtained by introducing into the packaging cells obtained according to the methods disclosed herein, a recombinant **retroviral vector** and propagating the resulting producer cells under conditions favorable for the production and secretion of **retroviral vector** supernatant. The retroviral supernatant produced by these methods also is claimed herein. This invention further provides a method for screening **retroviral vector** supernatant for high transduction efficiency and methods for producing **retroviral vector** supernatant for transducing cells with high efficiency in gene therapy applications.

CLM What is claimed is:

1. A method for obtaining a recombinant retroviral packaging cell comprising: a. isolating a retroviral nucleic acid sequence encoding a minimal **gag-pol** open reading frame (ORF), said nucleic acid sequence having no flanking sequences of the **gag-pol** ORF, and inserting said nucleic acid sequence into a first expression plasmid; b. isolating a retroviral nucleic acid sequence encoding a minimal env ORF, said nucleic acid sequence having no flanking sequences of the env ORF, and inserting said nucleic acid sequence into a second expression plasmid, c. obtaining a eukaryotic cell free of an endogenous nucleic acid sequence which encodes the **gag-pol** ORF or the env ORF and which is derived from the retrovirus from which the minimal **gag-pol** ORF or env ORF is isolated; and d. introducing the first and second expression plasmids into the eukaryotic cell and expressing the nucleic acids encoding the minimal **gag-pol** ORF and env ORF to produce **Gag**, **Pol** and Env proteins, thereby producing the recombinant retroviral packaging cell.

2. The method of claim 1, wherein the retrovirus is a murine leukemia virus.

3. The method of claim 1, wherein the cell is a non-murine cell.

4. The method of claim 3, wherein the non-murine cell is a primate cell.

5. The method of claim 4, wherein the primate cell is a human cell.

6. The method of claim 3, wherein the non-murine cell is selected from the group consisting of Vero, HT-1080, D17 MRC-5, TE671, human embryonic kidney, and HeLa cells.

7. The method of claim 6, wherein the human embryonic kidney cells are human 293 cells (ATCC CRL 1573).

8. The method of claim 1, wherein the **gag-pol** ORF is a **Moloney**

~~murine leukemia virus gag-pol~~ gene.

9. The method of claim 1, wherein the env ORF is a murine leukemia virus env gene.

10. The method of claim 1, wherein at least one of the expression plasmids further comprises a selectable or detectable marker gene.

11. The method of claim 1, wherein the screening is done by ELISA.

12. The method of claim 11, wherein in the ELISA, Env is detected using a primary antibody from hybridoma 83A25 followed by antiserum 79S-834, enzyme-conjugated antispecies antibody and enzyme substrate; and **Gag** is detected separately using a primary antibody from hybridoma R187 followed by antiserum 77S-227, enzyme-conjugated antispecies antibody and enzyme substrate.

13. The method of claim 8 wherein the **gag-pol** gene is expressed from the MMLV-LTR promoter.

14. The method of claim 8, wherein the **gag-pol** gene is expressed from the CMV-IE promoter or the RSV-LTR promoter.

15. The method of claim 1 wherein the **gag-pol** ORF and env ORF are isolated from the same retrovirus.

16. The method of claim 1, wherein the **gag-pol** ORF and env ORF are isolated from different retroviruses.

17. The method of claim 1, wherein the first and second expression plasmids are introduced into the eukaryotic cell in separate and sequential steps.

18. The method of claim 1, further comprising screening the cell of step (d) for retroviral **Gag**, **Pol** and Env production.

19. The recombinant retroviral packaging cell obtained by the method of claim 1.

20. The retroviral packaging cell of claim 19, wherein the cell produces an amphotropic Env.

21. The retroviral packaging cell of claim 19, wherein the cell produces a xenotropic Env.

22. The retroviral packaging cell of claim 19, wherein the cell produces a chimeric amphotropic/xenotropic Env.

23. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is a non-murine cell.

24. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is derived from human 293 having ATCC Accession No. CRL 1573.

25. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is a primate cell.

26. A method of producing a **retroviral vector** producer cell which comprises transducing the cells of claim 19 or 23 with a retroviral-based vector and subsequently propagating the cell under conditions favorable for the production and secretion of **retroviral vector** supernatant.

27. The method of claim 26, further comprising screening the producer cell for the ability to produce a vector supernatant having high

transduction efficiency, comprising modulating the ability of the vector supernatant to transduce a target cell population with a transduction efficiency greater than that achieved with a vector supernatant produced from murine PA317-based cells.

28. The method of claim 27, wherein the target cell population is human 293 cells (ATCC CRL 1573).

29. The method of claim 26, wherein the retroviral-based vector used to transduce the cells was produced in human cells.

30. The **retroviral vector** producer cell produced by the method of claim 26.

31. A method of increasing the gene transduction efficiency of a cell, comprising transducing the cell with a **retroviral vector** supernatant produced from the culture of at least one **retroviral vector** producer cell of claim 30, wherein the transduction efficiency is increased over that achieved with a vector supernatant produced from murine PA317-based cells.

32. The method of claim 31, wherein the **retroviral vector** producer cell is derived from a packaging cell selected from group consisting of ProPak-A.6 (ATCC Accession No. CRL 12006), ProPak-A.52 (ATCC Accession No. CRL-12479) or ProPak-X.36 (ATCC Accession No. CRL 12007).

33. The method of claim 31 wherein the **retroviral vector** supernatant is produced from the co-culture of a first and a second complementary **retroviral vector** producer cell without replication competent retrovirus generation.

34. The method of claim 30 wherein the first **retroviral vector** producer cell is derived from an amphotropic packaging cell and the second vector producer cell is derived from a xenotropic packaging cell.

35. The method of claim 34 wherein the amphotropic packaging cell is ProPak-A.6 (ATCC Accesssion No. CRL 12006) or ProPak-A.52 (ATCC Accession No. CRL-12479), and the xenotropic packaging cell is ProPak-X.36 (ATCC Accesssion No. CRL 12007).

36. The method of claim 34 wherein both the amphotropic and xenotropic packaging cells are produced from human 293 cells (ATCC CRL 1573).

37. The method of claim 31 wherein the **retroviral vector** supernatant is produced from a stable **retroviral vector** producer cell culture.

38. The method of claim 31 wherein the transduced cell is a primary human hematopoietic cell.

39. The method of claim 31 wherein the transduced cell is a human hematopoietic stem cell.

40. The method of claim 38 wherein the hematopoietic cell is a CD34+ Thy1+ cell from mobilized peripheral blood or a CD4+ PBL.

41. The retroviral packaging cell of claim 19, capable of packaging **retroviral vector** sequences to form a **retroviral vector** producer cell that does not generate RCR after continuous culture for up to at least 12 weeks and that produces a recombinant, transducing **retroviral vector** particle, the **retroviral vector** particle characterized by: a. being resistant to human complement; and b. having a high transduction efficiency.

42. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak-A.6 and has ATCC Accesssion No. CRL 12006.

43. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak A.52 having ATCC Accession No. CRL-12479.

44. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak-X.36 and has ATCC Accession No.CRL 12007.

45. An expression plasmid for expressing **gag-pol**, comprising a **gag-pol** open reading frame from the start codon to the stop codon with no flanking sequences of the open reading frame.

46. An expression plasmid for expressing env, comprising an env open reading frame from the start codon to the stop codon with no flanking sequences of the open reading frame.

L25 ANSWER 13 OF 21 USPATFULL on STN
1999:141681 Recombinant hepatitis virus vectors.
Tyrrell, D. Lorne J., 121 Laurier Drive, Edmonton, Alberta, Canada T5R5P6
Chaisomchit, Sumonta, 01A-8903, 112 Street, Edmonton, Alberta, Canada T6G2C5
Chang, Lung-Ji, 11615, 72 Avenue, Edmonton, Alberta, Canada T6G0B9
US 5981274 19991109
APPLICATION: US 1996-715808 19960918 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to recombinant hepatitis viral vectors useful for the expression of functional heterologous gene products in liver cells. It is contemplated that these vectors will find use in anti-viral, anti-tumor and/or gene therapy, particularly for the correction of inherited single-gene defects. These novel recombinant vectors may be used to deliver genes to cells in vivo by a variety of means including infection and direct injection of vector DNA.
CLM What is claimed is:
1. A recombinant hepatitis B virus genome comprising heterologous gene sequences which express at least one functional heterologous gene product.

2. The recombinant virus genome of claim 1, wherein said genome further comprises an endogenous viral promoter.

3. The recombinant virus genome of claim 1, wherein said viral promoter is selected from the group consisting of the core/**pol** promoter and the preS1 promoter.

4. The recombinant virus genome of claim 1, wherein said genome further comprises a heterologous promoter.

5. The recombinant virus genome of claim 4, wherein said heterologous promoter is selected from the group consisting of the CMV-IE promoter, the human elongation factor 1α gene promoter, the SV40 enhancer/promoter, the Rous sarcoma virus long terminal repeat, the α-fetoprotein gene promoter and the recombinant **Moloney murine leukemia virus** long terminal repeat containing CMV-IE/HIV-1 TAR sequences listed in SEQ ID NO:16.

6. The recombinant virus genome of claim 1, wherein said genome is replication competent.

7. The recombinant virus genome of claim 1, wherein said genome is replication defective.

8. A host cell transfected with a recombinant hepatitis B virus genome comprising **pol** gene sequences, X gene sequences and preS1/preS2/S gene sequences and heterologous gene sequences wherein said host cell expresses at least one functional heterologous gene product.

9. The host cell of claim 8, wherein said genome is replication defective.

10. The host cell of claim 9, wherein said genome contains a deletion within the **pol** gene.

11. The host cell of claim 9, wherein said genome contains a deletion within the preS/preS2/S gene sequences.

12. The host cell of claim 9, wherein said genome contains a deletion within the **pol** gene and the preS/preS2/S gene sequences.

13. The host cell of claim 8, wherein said genome lacks a functional X gene.

14. The host cell of claim 8, wherein said genome lacks a functional S gene.

15. The host cell of claim 14, wherein said genome further lacks functional preS1/S and preS2/S genes.

16. The host cell of claim 8, wherein said genome lacks a functional S gene and a functional X gene.

17. A method for encapsidating a recombinant hepatitis B virus genome, comprising: a) providing: i) a recombinant hepatitis B virus genome comprising **pol** gene sequences, X gene sequences and preS1/preS2/S gene sequences and heterologous gene sequences wherein said recombinant genome is capable of expressing at least one functional heterologous gene product and wherein said recombinant genome lacks the ability to produce at least one viral product required for packaging said recombinant genome; ii) at least one plasmid capable of providing in trans hepatitis B virus gene products sufficient to complement said recombinant viral genome lacking the ability to produce at least one viral product required for packaging; iii) a liver cell in vitro; and b) introducing said recombinant hepatitis virus genome and said at least one plasmid into said liver cell under conditions such that said recombinant hepatitis virus genome is encapsidated into viral particles.

18. The method of claim 17, wherein said liver cell is selected from the group consisting of human liver cells, avian liver cells, non-human primate liver cells, and rodent liver cells.

19. The method of claim 17, wherein said recombinant virus genome contains a deletion within the **pol** gene.

20. The method of claim 19, wherein said plasmid encodes the product of the hepatitis B virus **pol** gene.

21. The method of claim 17, wherein said recombinant virus genome contains a deletion within the preS/preS2/S gene sequences.

22. The method of claim 21, wherein said plasmid encodes the products of the hepatitis B virus preS/preS2/S gene sequences.

23. The method of claim 17, wherein said recombinant virus genome contains a deletion within the **pol** gene and the preS/preS2/S gene sequences.

24. The method of claim 17, wherein said plasmid encodes the products of the hepatitis B virus preS/preS2/S gene sequences and the product of the hepatitis B virus **pol** gene.

25. The method of claim 17, wherein said recombinant virus genome lacks a functional X gene.

L25 ANSWER 14 OF 21 USPATFULL on STN
1999:128827 Pantropic **retroviral vectors** for gene transfer in mollusks.
Burns, Jane C., La Jolla, CA, United States
Chen, Thomas T., Storrs, CT, United States
Ther Regents of The University of California, Oakland, CA, United States (U.S. corporation)The University of Conneticut, Farmington, CT, United States (U.S. corporation)
US 5969211 19991019
APPLICATION: US 1997-844530 19970418 (8) <--
PRIORITY: US 1996-16253P 19960419 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for introducing foreign nucleic acid sequences into marine mollusks. A pantropic **retroviral vector** containing a foreign gene sequence is introduced into fertilized mollusk embryos by electroporation. The gene sequence becomes integrated into the host DNA and encodes a functional protein product. This method has implications in the introduction of disease-resistance and growth-accelerating genes into mollusks.

CLM What is claimed is:

1. A method for introducing exogenous DNA into a germline of a marine mollusk, comprising the steps of: inserting said exogenous DNA into a plasmid comprising a promoter that is active in mollusks and retroviral long terminal repeats (LTRs), wherein said exogenous DNA is operably linked to said promoter, thereby generating a recombinant vector; forming a pseudotyped retrovirus containing VSV G protein and RNA corresponding to said recombinant vector; and infecting a mollusk embryo with said pseudotyped retrovirus so as to insert said exogenous DNA into the germline of said mollusk.

2. The method of claim 1, wherein said marine mollusk is a clam.

3. The method of claim 1, wherein said marine mollusk is selected from the group consisting of oyster, mussel, scallop and abalone.

4. The method of claim 1, wherein said retroviral long terminal repeats are **Moloney murine leukemia virus** long terminal repeats.

5. The method of claim 1, wherein said infecting step is performed by electroporation.

6. The method of claim 1, wherein said infecting step is performed by dechorionation or microinjection.

7. The method of claim 1, wherein the inserting step comprises inserting said exogenous DNA into a first plasmid containing retroviral long terminal repeats, a gene encoding a selectable marker, and said promoter is operably linked to said exogenous DNA.

8. The method of claim 7, wherein the forming step comprises: transfecting a packaging cell line with said first plasmid, wherein said cell line assembles vector particles containing an RNA copy of said recombinant vector; infecting a producer cell line with said vector particles, said producer cell line containing retroviral **gag** and **pol**; transfecting said vector particle-containing producer cell line with a second plasmid containing a promoter operably linked to VSV G protein, whereby pseudotyped **retroviral vectors** containing the VSV-G protein are secreted by said producer cell line.

9. The method of claim 7, wherein said first plasmid is pLSRNL or pGeo4.8.

10. The method of claim 7, wherein said promoter is the Rous sarcoma virus promoter.

11. The method of claim 7, wherein said selectable marker is neomycin phosphotransferase.

12. The method of claim 8, wherein said packaging cell line is PA317.

13. The method of claim 8, wherein said producer cell line is 293 cells.

14. The method of claim 8, wherein said second plasmid is pHCMV-G.

15. The method of claim 8, wherein said promoter of said second plasmid is cytomegalovirus promoter.

L25 ANSWER 15 OF 21 USPATFULL on STN
1999:65191 Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant.
Rigg, Richard J., Mountain View, CA, United States
Chen, Jingyi, Fremont, CA, United States
Dando, Jonathan S., Sunnyvale, CA, United States
Plavec, Ivan, Menlo Park, CA, United States
Forestell, Sean P., Menlo Park, CA, United States
Bohnlein, Ernst, Los Altos, CA, United States
SyStemix, Inc., Palo Alto, CA, United States (U.S. corporation)
US 5910434 19990608
APPLICATION: US 1995-572959 19951215 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** and the recombinant packaging cell obtained by the method. Also provided is a method of producing recombinant retroviral particles obtained by introducing into the packaging cells obtained according to the methods disclosed herein, a recombinant **retroviral vector** and propagating the resulting producer cells under conditions favorable for the production and secretion of **retroviral vector** supernatant. The retroviral supernatants produced by these methods also is claimed herein. This invention further provides a method for screening **retroviral vector** supernatant for high transduction efficiency and methods for producing **retroviral vector** supernatant for transducing cells with high efficiency in gene therapy applications.

CLM What is claimed is:

1. A method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** comprising: a. selecting a retrovirus; b. obtaining a eukaryotic cell free of endogenous retroviral nucleic acid of the same type as the retrovirus of step (a); c. preparing a minimal **gag-pol** open reading frame (ORF) insert from the retrovirus wherein the ORF contains no flanking sequences of the **gag-pol** gene; d. inserting the minimal **gag-pol** ORF prepared from step (c) into an appropriate expression plasmid, wherein the **gag-pol** ORF is operatively linked to a heterologous promoter having no overlap with the **retroviral vector**; e. preparing a minimal env open reading frame (ORF) insert from the retrovirus wherein the ORF contains no flanking sequences of the env gene; f. inserting the minimal env ORF prepared from step (e) into an appropriate expression plasmid, wherein the env ORF is operatively linked to a heterologous promoter having no overlap with the **retroviral vector**; g. inserting the expression plasmids of steps (d) and (f) into the cell of step (b); h. propagating the cell obtained from step (g) under conditions favorable for expression of the minimal retroviral **gag-pol** and env ORF; and i. screening a cell for retroviral **Gag**, **Pol** and Env production by the cell of step (h); thereby obtaining the retroviral packaging cell capable of packaging recombinant **retroviral vector** sequences to produce recombinant, transducing retrovirus.

2. The method of claim 1, wherein the retrovirus is a **Moloney murine leukemia virus**.

3. The method of claim 1, wherein the cell is a non-murine cell.

4. The method of claim 3, wherein the non-murine cell is a primate cell.

5. The method of claim 4, wherein the primate cell is a human cell.

6. The method of claim 3, wherein the non-murine cell is selected from the group consisting of Vero, HT-1080, D17 MRC-5, FS-4, TEG71, human embryonic kidney (293), and HeLa.

7. The method of claim 6, wherein the human embryonic kidney cells are 293 cells (ATCC CRL 1573).

8. The method of claim 1, wherein the **gag-pol** ORF is a **moloney murine leukemia virus gag-pol** gene.

9. The method of claim 8, wherein the **gag-pol** gene is expressed from the CMV-IE promoter or the RSV-LTR promoter.

10. The method of claim 1, wherein the env ORF is a **moloney murine leukemia virus** env gene.

11. The method of claim 1, wherein the plasmid of steps (d) or (f) comprises a selectable or detectable marker gene.

12. The method of claim 1, wherein the **gag-pol** ORF and the env ORF expression plasmids of step (g) are amplified in bacterial host cells prior to inserting into the eukaryotic cell of step (b), wherein the bacterial host cells are propagated at a temperature range from about 28° C. to about 32° C.

13. The method of claim 12, wherein the bacterial host cells are propagated at about 30° C.

14. The method of claim 1, wherein in step (i), **Gag**, **Pol** and Env production is screened by a sandwich ELISA assay.

15. The method of claim 14, wherein Env is detected using a primary antibody from hybridoma 83A25 followed by antiserum 79S-834, enzyme-conjugated antispecies antibody and enzyme substrate; and **Gag** is detected separately using a primary antibody from hybridoma R187 followed by antiserum 77S-227, enzyme-conjugated antispecies antibody and enzyme substrate.

16. The recombinant retroviral packaging cell obtained by the method of claim 1.

17. The recombinant retroviral packaging cell of claim 16, wherein the cell produces an amphotropic env.

18. The recombinant retroviral retroviral packaging cell of claim 16, wherein the cell produces a xenotropic env.

19. A retroviral packaging cell line designated ProPak-A.6 having ATCC Accession No. CRL 12006.

20. A method of producing a retroviral producer cell which comprises transducing the cells of claim 16 with a retroviral-based vector and subsequently propagating the cells under conditions favorable for the production and secretion of **retroviral vector** supernatant.

21. The method of claim 20, further comprising screening the producer cell for the ability to produce a vector supernatant having high transduction efficiency, comprising measuring the ability of the vector supernatant to transduce a target cell population with a transduction

efficiency greater than that achieved with a vector supernatant produced from murine PA317-based cells.

22. The method of claim 21, wherein the target cell population is human 293 cells.

23. The retroviral producer cell produced by the method of claim 20.

L25 ANSWER 16 OF 21 USPATFULL on STN
1999:4402 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Roberts, Margo R., San Francisco, CA, United States
Dull, Thomas J., San Francisco, CA, United States
Zsebo, Krisztina M., Woodside, CA, United States
Qin, Lu, Foster City, CA, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 5858740 19990112
APPLICATION: US 1995-438582 19950510 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging constructs and packagable vector transcripts are produced from high expression plasmids by transfection in human cells. High titers of recombinant retrovirus are produced in infected cells. The methods of the invention include the use of the novel retroviral constructs to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation at high efficiencies. The invention is useful for the rapid production of high viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.
CLM What is claimed is:
1. A mammalian target cell transduced with **retroviral vector** pDRTD4.2 comprising in the 5' to 3' direction, a modified 5' **Moloney murine leukemia virus** (MMLV) long terminal repeat (LTR) region comprising replacement of the U3 region of the 5' LTR with the U3 region of Moloney murine sarcoma virus (MMSV) viral **gag** sequences up to the Nar I site of MMLV, a retroviral splice acceptor, a 3' MMLV LTR region and a foreign gene inserted downstream of said splice acceptor.

2. The target cell of claim 1, wherein said foreign gene encodes a growth factor, a lymphokine, a hormone or a coagulation factor.

3. The target cell of claim 1, wherein said foreign gene encodes a chimeric T cell receptor.

4. The target cell of claim 3, wherein said chimeric T cell receptor is encoded by a DNA comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

5. The target cell of claim 4, wherein said cytoplasmic domain is selected from the group consisting of the CD3 zeta chain, the CD3 eta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain.

6. The target cell of claim 4, wherein said cytoplasmic domain is the gamma chain of the FcεR1 receptor.

7. The target cell of claim 4, wherein said extracellular domain is a single-chain antibody, or antigen-binding portion thereof.

8. The target cell of claim 4, wherein said extracellular domain is a single-chain antibody specific for the HIV env glycoprotein and said cytoplasmic domain is zeta.

9. The target cell of claim 4, wherein said chimeric T cell receptor is a CD4/zeta receptor.

10. The target cell of claim 6, wherein said extracellular domain is a CD antigen.

11. The target cell of claim 10, wherein said extracellular domain is CD4 or CD8.

12. The target cell of claim 7, wherein said single-chain antibody is specific for the HIV env glycoprotein.

13. A method for transducing with high efficiency mammalian target cells with foreign genes, said method comprising: A) culturing stably transfected 293 cells producing replication-defective recombinant **retroviral vectors** carrying a selected foreign gene; B) separating said transfected 293 cells from supernatant; and C) exposing said mammalian target cells to said supernatant, whereby target cells efficiently transduced with said foreign gene are obtained.

14. The method of claim 13, wherein said mammalian target cells are human cells.

15. The method of claim 14, wherein said human cells are lymphocytes.

16. The method of claim 14, wherein said human cells are hematopoietic stem cells.

17. A mammalian target cell transduced with replication-defective **retroviral vector** pRTD2.2 comprising in the 5' to 3' direction, a modified 5' MMLV LTR region comprising replacement of the 5' LTR with the human cytomegalovirus CMV) immediate early enhancer/promoter fused to the MMLV R region by an oligonucleotide encoding nucleotides 19 (Sac I) to +1 of the human CMV promoter linked to nucleotides +1 to +32 (KpnI) of MMLV, viral **gag** sequences up to the Nar I site of MMLV, a retroviral splice acceptors, an MMLV 3' LTR region and a foreign gene inserted downstream of said splice acceptor.

18. A mammalian target cell transduced with replication-defective **retroviral vector** pRTD2.2SVG comprising a modification of the pRTD2.2 vector of claim 17 consisting of replacement of the Sac I to Bst EII fragment of vector pRTD2.2 with the Sac I to Bst EII fragment of vector LXSN.

19. A mammalian target cell transduced with replication-defective **retroviral vector** pIKT2.2 comprising a modification of the pIK1.1 vector containing the SV40 T antigen polyadenylation site and the SV40 origin of replication, said modification consisting of insertion of the DNA sequence between the 5' LTR and 3' LTR of vector pRTD2.2 of claim 17 between the Sac I and Eco RI sites of pIK1.1.

20. A mammalian target cell transduced with replication-defective **retroviral vector** pIKT2.2 comprising a modification of the pIK1.1 vector containing the SV40 T antigen polyadenylation site and the SV40 origin of replication, consisting of insertion of the DNA defined at its 5' end by the Sac I site in the human CMV promoter and defined at its 3' end by an Eco RI site located approximately 750 bp downstream of the 3' LTR of vector pRTD2.2SVG of claim 18, between the Sac I and Eco RI sites of pIK1.1.

21. A mammalian target cell transduced with a replication-defective

retroviral vector comprising (a) a modification of the pIK1.1 vector in which the sequences of pIK1.1 downstream of the human CMV immediate early enhancer/promoter and upstream of the SV40 origin of replication and SV40 polyadenylation site are replaced with a fragment of a first **retroviral vector** consisting of the 5' R region of the first **retroviral vector** up to a restriction site downstream of the 3' LTR of said first **retroviral vector**, wherein said vector comprises a splice acceptor, and (b) a foreign gene inserted downstream of said splice acceptor.

22. A mammalian target cell transduced with the replication-defective **retroviral vector** of claim 21, wherein said first **retroviral vector** is an MMLV vector.

23. The target cell of claim 1, 17, 18, 19, 20, 21 or 22, wherein said cell is a human cell.

24. The target cell of claim 1, 17, 18, 19, 20, 21 or 22, wherein said cell is selected from the group consisting of lymphocyte, human hematopoietic stem cell, fibroblast, epithelial cell, endothelial cell, myoblast, retinal epithelial cell, islet of Langerhans cell, adrenal medulla cell, osteoblast, osteoclast, neuron, glial cell, ganglion cell, embryonic stem cell and hepatocyte.

25. The mammalian target cell of claim 1, 17, 18, 19, 20, 21 or 22, wherein the splice receptor is replaced with a transcriptional control element internal to the vector selected from the group consisting of a promoter, enhancer, enhancer/promoter and a dominant control region.

26. The mammalian target cell of claim 17, 18, 19, 20, 21 or 22, wherein said foreign gene encodes a chimeric T cell receptor.

27. A method for transducing with high efficiency mammalian target cells with foreign genes, said method comprising cocultivating transfected human cells producing replication-defective recombinant **retroviral vectors** carrying a selected foreign gene with mammalian target cells, wherein said vectors transduce said target cells with said foreign gene.

28. The method of claim 27, wherein said transfected human cells are transiently cotransfected.

29. The method of claim 27, wherein said transfected human cells are stably cotransfected.

30. The method of claim 27, wherein said mammalian target cells are human cells.

31. The method of claim 30, wherein said human cells are lymphocytes.

32. The method of claim 30, wherein said human cells are hematopoietic stem cells.

33. A method for transducing with high efficiency mammalian target cells with foreign genes, said method comprising: A) culturing transfected human cells producing replication-defective recombinant **retroviral vectors** carrying a selected foreign gene; B) separating said transfected human cells from supernatant; and C) exposing said mammalian target cells to said supernatant, whereby target cells efficiently transduced with said foreign gene are obtained.

34. The method of claim 33, wherein said transfected human cells are transiently cotransfected.

35. The method of claim 33, wherein said transfected human cells are stably cotransfected.

36. The method of claim 35, wherein said mammalian target cells are human cells.

37. The method of claim 36, wherein said human cells are lymphocytes.

38. The method of claim 36, wherein said human cells are hematopoietic stem cells.

39. A cell stably transfected with a retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans the **gag** and **pol** virion proteins for packaging a replication-incompetent **retroviral vector** and for producing virion proteins capable of packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and containing an SV40 polyadenylation site.

40. The cell of claim 39 wherein said cell is a human cell.

41. The mammalian target cell of claim 26, wherein said receptor is a CD4/zeta or single-chain antibody chain/zeta T cell receptor.

42. A method for transducing mammalian target cells with foreign genes, said method comprising: A) transiently transfecting a first population of mammalian cells that produce virus, wherein the first population of mammalian cells are stably transfected with at least two retroviral packaging plasmids, which are transfected sequentially, comprising retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and including an SV40 polyadenylation site with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; and B) cocultivating said first population of mammalian cells producing replication-defective recombinant **retroviral vectors** carrying said foreign gene with a second population of mammalian target cells, wherein said recombinant **retroviral vectors** transduce said second population of target cells with said foreign gene.

43. A method for transducing mammalian target cells with foreign genes, said method comprising: A) transiently transfecting a first population of mammalian cells that produce virus, wherein the first population of mammalian cells are stably transfected with at least two retroviral packaging plasmids, which are transfected sequentially, comprising retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and including an SV40 polyadenylation site with a **retroviral vector** encoding a foreign

gene to produce replication defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; and B) separating said first population of mammalian cells from supernatant containing recombinant **retroviral vectors**; and C) incubating said supernatant with a second population of mammalian target cells, wherein said recombinant **retroviral vector** transduces said second population of target cells with said foreign gene.

44. A human cell stably transfected with a retroviral packaging plasmid for producing high titers of recombinant retrovirus in human target cells comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins which package said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging the helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in said human cell and a SV40 polyadenylation site.

45. The human cell of claim 44 which is an embryonic kidney cell.

46. The human cell of claim 44, wherein said human cell comprises two retroviral helper DNA sequences.

47. The human cell of claim 45, wherein said embryonic kidney cell is a 293 cell.

48. The human cell of claim 47, wherein said 293 cell is a tsa54 cell.

49. The human cell of claim 46, wherein a first helper sequence codes for human immunodeficiency virus (HIV) or gibbon ape leukemia virus (GALV) **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus (VSV), human T cell leukemia virus (HTLV) type I and HTLV type II.

50. The human cell of claim 49, wherein said first helper sequence codes for HIV **gag** and **pol** proteins.

L25 ANSWER 17 OF 21 USPATFULL on STN
1998:138699 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Roberts, Margo R., San Francisco, CA, United States
Dull, Thomas J., San Francisco, CA, United States
Zsebo, Krisztina M., Woodside, CA, United States
Qin, Lu, Foster City, CA, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 5834256 19981110
APPLICATION: US 1993-76299 19930611 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging constructs and packagable vector transcripts are produced from high expression plasmids by transfection in human cells. High titers of recombinant retrovirus are produced in infected cells. The methods of the invention include the use of the novel retroviral constructs to transduce primary human cells, including T cells and bone

marrow stem cells, with foreign genes by cocultivation at high efficiencies. The invention is useful for the rapid production of high viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.

CLM What is claimed is:

1. A method for transducing, mammalian target cells with foreign genes, said method comprising: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) at least one retroviral packaging plasmid comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer without the production of replication competent helper virus said retroviral helper DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a SV40 polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; and B) cocultivation of said first population of mammalian cells producing replication-defective recombinant **retroviral vectors** carrying said foreign gene with a second population of mammalian target cells, to transduce said second population of target cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

2. The method of claim 1, wherein said target cells are selected from the group consisting of lymphocytes, human hematopoietic stem cells, fibroblasts, epithelial cells, endothelial cells, myoblasts, retinal epithelial cells, islets of Langerhans, adrenal medulla cells, osteoblasts, osteoclasts, neurons, glial cells, ganglion cells, embryonic stem cells, and hepatocytes.

3. The method of claim 1, wherein said population of mammalian target cells are human cells.

4. The method of claim 1, wherein said population of mammalian target cells are human hematopoietic stem cells.

5. The method of claim 1, wherein said first population of mammalian cells are human embryonic kidney cells.

6. The method of claim 1, wherein said retroviral genome is a leukemia viral genome selected from the group consisting of **Moloney murine leukemia virus** (MMLV), Human immunodeficiency virus (HIV) and Gibbon ape leukemia virus (GALV).

7. The method of claim 1, wherein said retroviral packaging plasmid comprises two retroviral helper DNA sequences.

8. The method of claim 1, wherein said foreign gene is selected from the group consisting of gene coding growth factors, lymphokines, hormones and coagulation factors.

9. The method of claim 1, wherein said foreign gene encodes a chimeric T cell receptor.

10. The method of claim 3 wherein said human target cells are lymphocytes.

11. The method of claim 10, wherein said lymphocytes are T cells.

12. The method of claim 10, wherein said lymphocytes are selected from the group consisting of CD8 positive cytotoxic T cells, CD4 positive T cells and tumor-infiltrating lymphocytes.

13. The method of claim 11, wherein said T cells are cytotoxic T cells.

14. The method of claim 5, wherein said human embryonic kidney cells are 293 cells.

15. The method of claim 14 wherein said 293 cells are tsa201 cells.

16. The method of claim 6, wherein said foreign enhancer is the human cytomegalovirus (CMV) immediate early enhancer and said promoter is the native MMLV promoter.

17. The method of claim 6, wherein said foreign enhancer and promoter is the human CMV immediate early enhancer and promoter.

18. The method of claim 6, wherein said foreign enhancer and promoter is the Moloney murine sarcoma virus (MMSV) enhancer and promoter.

19. The method of claim 7, wherein a first helper sequence codes for ecotropic MMLV **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus (VSV), human T cell leukemia virus (HTLV) type I and HTLV type II.

20. The method of claim 7 wherein a first helper sequence codes for HIV **gag** and **pol** proteins or GALV **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus, human T cell leukemia virus (HTLV) type I and HTLV type II.

21. The method of claim 9, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

22. The method of claim 21, wherein said cytoplasmic domain is selected from the group consisting of gene coding the CD3 zeta chain, the eta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain.

23. The method of claim 21, wherein said cytoplasmic domain is the gamma chain of the FcεR1 receptor.

24. The method of claim 21, wherein said extracellular domain is a single-chain antibody, or functional portion thereof.

25. The method of claim 21, wherein said extracellular domain is a single-chain antibody specific for the HIV env glycoprotein and said cytoplasmic domain is zeta.

26. The method of claim 21, wherein said chimeric T cell receptor is a CD4/zeta receptor.

27. The method of claim 23, wherein said extracellular domain is a CD antigen.

28. The method of claim 27, wherein said extracellular domain is CD4 or

29. The method of claim 24, wherein said single-chain antibody is specific for the HIV env glycoprotein.

30. A method for transducing mammalian target cells with foreign genes, said method comprising: A) transient cotransfection of 293 cells with (i) at least one retroviral packaging plasmid comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and promoter functional in a selected mammalian cell and a SV40 polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said 293 cells; and B) cocultivation of said 293 cells producing replication-defective recombinant **retroviral vectors** carrying said foreign gene with a second population of mammalian target cells, to transduce said population of target cells with said foreign gene, whereby target cells efficiently transduced with said foreign gene are obtained.

31. The method of claim 30, wherein said target cells are human target cells.

32. The method of claim 31, wherein said human target cells are lymphocytes.

33. The method of claim 31, wherein said human target cells are hematopoietic stem cells.

34. A method for transducing mammalian target cells with foreign genes, said method comprising cocultivation of transfected 293 cells producing replication-defective recombinant **retroviral vectors** carrying a selected foreign gene with mammalian target cells, to transduce said target cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

35. The method of claim 34, wherein said 293 cells are transiently cotransfected.

36. The method of claim 34, wherein said 293 cells are stably transfected.

37. The method of claim 34, wherein said mammalian target cells are human cells.

38. The method of claim 34, wherein said 293 cells are transiently cotransfected with: (a) at least one retroviral packaging plasmid comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and promoter functional in a selected mammalian cell and a SV40 polyadenylation site; and (b) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said 293 cells.

39. The method of claim 37, wherein said human cells are lymphocytes.

40. The method of claim 37, wherein said human cells are hematopoietic stem cells.

41. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and promoter functional in a selected mammalian cell and a SV40 polyadenylation site.

42. The retroviral packaging plasmid of claim 41, wherein said retrovirus is a leukemia retrovirus.

43. The retroviral packaging plasmid according to claim 41 wherein said helper DNA sequence codes for ecotropic MMLV **gag** and **pol**, and an envelope protein, or combination thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus, human T cell leukemia virus (HTLV) type I and HTLV type II.

44. A method for transiently producing replication-defective recombinant retrovirus in mammalian cells at high titer comprising introducing into mammalian cells that can produce virus at least one retroviral packaging plasmid according to claim 41 and a **retroviral vector** encoding a foreign gene, whereby mammalian cells containing said retroviral packaging plasmid and **retroviral vector** produce high titers of retrovirus for infection.

45. The retroviral packaging plasmid of claim 42, wherein said leukemia retrovirus is selected from the group consisting of Moloney murine leukemia viruses (MMLV), Gibbon ape leukemia viruses (GALV), and HIV viruses.

46. The retroviral packaging plasmid of claim 45, wherein said foreign enhancer is the human CMV immediate early enhancer and said promoter is the native MMLV promoter.

47. The retroviral packaging plasmid of claim 45, wherein said foreign enhancer and promoter is the human CMV immediate early enhancer and promoter.

48. The retroviral packaging plasmid of claim 45, wherein said foreign enhancer and promoter is the MMSV enhancer and promoter.

49. The retroviral packaging plasmid of claim 45, wherein said plasmid comprises two retroviral helper DNA sequences.

50. The retroviral packaging plasmid of claim 49, wherein a first helper sequence codes for ecotropic MMLV **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus (VSV), human T cell leukemia virus (HTLV) type I and HTLV type II.

51. The method of claim 44 wherein said mammalian cells are human cells.

52. A transfected cell producing replication-defective recombinant retroviruses at high titer, said cell prepared by the method of claim 44.

53. The method of claim 51, wherein said human cells are human embryonic kidney cells.

54. The method of claim 53, wherein said human embryonic kidney cells are 293 cells.

55. The method of claim 54 wherein said 293 cells are tsa201 cells.

56. The transfected cell of claim 52, wherein said cell is a human cell.

57. The transfected cell of claim 56, wherein said human cell is a human embryonic kidney cell.

58. The transfected cell of claim 57, wherein said embryonic kidney cell is a 293 cell.

59. The transfected cells of claim 58 wherein said 293 cells are tsa201 cells.

60. A replication-defective **retroviral vector** comprising in the 5' to 3' direction, a modified 5' MMLV LTR region wherein the U3 region of the 5' LTR is replaced with the U3 region of MMSV, viral **gag** sequences up to the Nar I site of MMLV, a retroviral splice acceptor and a 3' MMLV LTR region.

61. A replication-defective **retroviral vector** comprising in the 5' to 3' direction, a modified 5' MMLV LTR region wherein the 5' LTR is replaced with the human CMV immediate early enhancer/promoter fused to the MMLV R region by an oligonucleotide encoding nucleotides 19 (Sac I) to +1 of the human CMV promoter linked to nucleotides +1 to +32 (KpnI) of MMLV, viral **gag** sequences up to the Nar I site of MMLV, a retroviral splice acceptor and a MMLV 3' LTR region.

62. A replication-defective **retroviral vector** comprising a modification of the vector of claim 61 wherein the Sac I to Bst EII fragment of the vector of claim 64 is replaced with the Sac I to Bst EII fragment of vector LXSN.

63. A replication-defective **retroviral vector** comprising a modification of pIK1.1 which contains the SV40 T antigen polyadenylation site and the SV40 origin of replication, wherein said modification consists of an insertion of the DNA sequence between the 5' LTR and 3' LTR of the vector of claim 61 between the SacI and EcoRI sites of pIK1.1.

64. A replication-defective **retroviral vector** comprising a modification of the pIK1.1 vector containing the SV40 T antigen polyadenylation site and the SV40 origin of replication, wherein the DNA, defined at its 5' end by the Sac I site in the human CMV promoter and defined at its 3' end by an Eco RI site located approximately 750 bp downstream of the 3' LTR of the vector of claim 62 is inserted, between the SacI and Eco RI sites of pIK1.1.

65. The **retroviral vector** of claim 64, wherein the splice acceptor is replaced with a transcriptional control element internal to the vector selected from the group consisting of a promoter, enhancer, enhancer/promoter and a dominant control region.

66. The **retroviral vector** of claim 60, 61, 62, 63 or 64 further comprising DNA encoding a foreign gene inserted downstream of said splice acceptor.

67. A replication-defective **retroviral vector** comprising a modification of pIK1.1 in which the sequences of pIK1.1 downstream of the human CMV immediate early enhancer/promoter and upstream of the SV40 origin of replication and SV40 polyadenylation site are replaced with a fragment of a first **retroviral vector** consisting of the 5' R region of the first **retroviral vector** up to a restriction site downstream of the 3' LTR of said first **retroviral vector**.

68. The replication-defective **retroviral vector** of claim 67, wherein said first **retroviral vector** is an MMLV vector.

69. The **retroviral vector** of claim 66 wherein said foreign gene encodes a chimeric T cell receptor.

70. The **retroviral vector** of claim 69 wherein said receptor is a CD4/zeta or single-chain antibody chain/zeta T cell receptor.

71. A method of using the replication-defective **retroviral vector** of claim 66 to express high levels of packagable genomic retroviral transcipts in mammalian cells comprising transiently cotransfecting a first population of mammalian cells with a packaging plasmid and said **retroviral vector** whereby said transcripts are produced.

72. A mammalian cell which produces recombinant retrovirus by the method of claim 71.

73. The method of claim 71, further comprising cocultivating said first population of mammalian cells with a second population of target cells to transduce said target cells with the foreign gene.

74. The mammalian cell according to claim 72, wherein said mammalian cell is a human cell.

75. The mammalian cell according to claim 74, wherein said human cell is a 293 cell.

76. The method of claim 73 wherein said target cells are lymphocytes.

77. A method for transducing mammalian target cells with foreign genes, said method comprising: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) at least one retroviral packaging plasmid comprising at least one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral helper DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a SV40 polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; and C) incubating of said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with a second population of mammalian target cells, to transduce said second population of target cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

78. The method of claim 77 wherein said first population of mammalian cells are human embryonic kidney cells.

79. The method of claim 77 wherein said retroviral packaging plasmid

comprises the retroviral helper DNA sequences.

80. The method of claim 78 wherein said human embryonic kidney cells are 293 cells.

81. The method of claim 79 wherein a first helper sequence codes for ecotropic MMLV **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus, human T cell leukemia virus (HTLV) type I and HTLV type II.

82. The method of claim 79 wherein a first helper sequence codes for HIV **gag** and **pol** proteins or GALV **gag** and **pol** proteins and a second helper sequence codes for env proteins, or combinations thereof, selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, Vesicular Stomatitis Virus, human T cell leukemia virus (HTLV) type I and HTLV type II.

83. The **retroviral vector** of claim 65, further comprising DNA encoding a foreign gene inserted downstream of said transcriptional control element.

84. The **retroviral vector** of claim 83, wherein said foreign gene is a chimeric T cell receptor.

85. A method of using the **retroviral vector** of claim 83 to express high levels of packagable genomic retroviral transcripts in mammalian cells which produce virus comprising transiently cotransfecting a first population of mammalian cells with a packaging plasmid and said **retroviral vector** whereby said transcripts are produced.

86. The **retroviral vector** of claim 84, wherein said receptor is a CD4/zeta or single-chain antibody/zeta T cell receptor.

87. The method of claim 85, further comprising cocultivating said first population of mammalian cells with a second population of target cells to transduce said target cells with the foreign gene.

88. A mammalian cell producing recombinant retroviruses produced by the method of claim 85.

89. The method of claim 87, wherein said target cells are lymphocytes.

90. Retroviral packaging plasmid pIK6.1MMSVampac, having the structure shown in FIG. 1.

91. Retroviral packaging plasmid pIK6.1MCVampac, having the structure shown in FIG. 1.

92. Retroviral packaging plasmid pIK6.1gagpolATG, having the structure shown in FIG. 1.

93. Retroviral packaging plasmid pIK6.1amenvATG, having the structure shown in FIG. 1.

94. The method of claim 1, wherein said retroviral packaging plasmid is the retroviral packaging plasmid of claim 90, 91, 92 or 93.

95. A **retroviral vector** designated pRTD4.2.

96. A **retroviral vector** designated pRTD2.2.

97. A **retroviral vector** designated pRTD2.2SVG.

98. A **retroviral vector** designated pIKT2.2.

99. A **retroviral vector** designated pIKT2.2SVG.

L25 ANSWER 18 OF 21 USPATFULL on STN
1998:122246 VSV G pseusdotyped **retroviral vectors**.
Yee, Jiing-Kuan, Del Mar, CA, United States
Emi, Nobuhiko, Nagoya, CA, United States
Friedmann, Theodore, La Jolla, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Barber, Jack R., San Diego, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)Chiron Viagene, Inc., Emeryville, CA, United States (U.S. corporation)
US 5817491 19981006
APPLICATION: US 1994-361839 19941222 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An enveloped vector particle contains gag and pol proteins from a retrovirus, a nucleic acid sequence and an envelope that includes VSV G envelope glycoprotein. The vector particle can be used to introduce nucleic acids into cells.
CLM What is claimed is:
1. An enveloped vector particle comprising: **gag** and **pol** proteins from a retrovirus; a nucleic acid sequence associated with said vector particle; an envelope having vesicular stomatitis virus (VSV) G envelope glycoprotein therein.

2. The vector particle of claim 1, wherein said **gag** and **pol** proteins are from the same species of retrovirus.

3. The vector particle of claim 2, wherein the nucleic acid sequence encodes a gene having an origin other than from said retrovirus, said gene being expressible into polypeptide.

4. The vector particle of claim 3, in which said polypeptide is a selectable marker.

5. The vector particle of claim 4, wherein the selectable marker is expressed by a neomycin resistance gene.

6. The vector particle of claim 3, wherein said nucleic acid sequence additionally comprises a promoter which can direct the transcription of said gene.

7. The vector particle of claim 2, wherein said **gag** and **pol** proteins are from **MoMLV**.

8. A method of introducing foreign nucleic acid into a cell, said method comprising infecting said cell with the enveloped vector particle of claim 1.

9. The method of claim 8, wherein said **gag** and **pol** proteins are from the same species of retrovirus.

10. The method of claim 9, wherein said cell is outside the normal host range of the retrovirus.

11. A method according to claim 9, wherein the retrovirus has a host range not including human cells or a subset of human cells.

12. A method according to claim 9, wherein the retrovirus is **MoMLV**.

13. A cell line that stably expresses or carries **gag/pol**, transfected or transduced with a nucleic acid molecule encoding a **retroviral vector** and a nucleic acid molecule encoding a VSV G protein, the cell line being capable of producing functional vector particles.

14. The cell line of claim 13, wherein the cell line is transiently transfected.

15. A 293 cell stably expressing **gag/pol**.

16. The cell of claim 15, wherein the cell is a 293 2-3 cell.

17. A method of making a VSV G pseudotyped virus, comprising: introducing into a cell line that stably expresses **gag/pol** a VSV G protein expression vector and a **retroviral vector** construct, the resulting cell line being capable of producing vector particles comprising VSV G protein.

18. The method of claim 17, wherein the **retroviral vector** construct is introduced prior to the VSV G protein expression vector.

19. The method of claim 18, wherein the method further comprises selecting for cells carrying the **retroviral vector** construct prior to introducing the VSV G protein expression vector.

20. A method of making a VSV G pseudotyped virus, comprising: (a) cotransfecting or sequentially transfecting a cell with a vector that directs the expression of **gag/pol** and a **retroviral vector** construct; and (b) introducing a VSV G protein expression vector into the cotransfected or sequentially transfected cell, the resulting cell being capable of producing vector particles comprising VSV G protein.

21. The method of claim 20, wherein the cell is cotransfected, and the method includes selecting for transfected cells prior to introducing a VSV G protein expression vector.

22. The method of claim 20, wherein the cell is sequentially transfected, and the method include selecting for cells expressing either **gag/pol** or the **retroviral vector** construct.

L25 ANSWER 19 OF 21 USPATFULL on STN
1998:115591 HIV-specific ribozymes.
Wong-Staal, Flossie, San Diego, CA, United States
Yu, Mang, San Diego, CA, United States
Yamada, Osamu, San Diego, CA, United States
Ojwang, Joshua O., Spring, TX, United States
Leavitt, Mark, La Jolla, CA, United States
Ho, Anthony, San Diego, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5811275 19980922
APPLICATION: US 1995-465483 19950605 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides an infectious retrovirus having inserted between the 5' and 3' long terminal repeat sequences of the retrovirus a nucleic acid encoding an anti-HIV-type specific agent under the control of a pol III promoter. Host cells containing the **retroviral vectors** of this invention also are provided. Further provided are methods of interfering with or preventing HIV viral replication in a cell infected with HIV or likely to be infected with HIV.
CLM What is claimed is:
1. A nucleic acid vector having two retroviral LTRs, and a nucleic acid

sequence encoding a ribozyme, wherein expression of said ribozyme is under the transcriptional control of a **pol** III promoter located between the two retroviral LTRs, and wherein the ribozyme specifically cleaves a human immunodeficiency virus nucleic acid.

2. The nucleic acid of claim 1, wherein the nucleic acid vector is a viral vector.

3. The nucleic acid of claim 1, wherein the nucleic acid vector is viral vector selected from the group of viral vectors consisting of an adeno associated viral vector, a **Moloney murine leukemia virus** vector, and a non-virulent vaccinia virus vector.

4. The nucleic acid of claim 1, wherein the nucleic acid vector is a retrovirus.

5. The nucleic acid of claim 1, wherein the nucleic acid vector is a retrotransposon.

6. The nucleic acid of claim 1, wherein the nucleic acid vector is a plasmid.

7. The nucleic acid of claim 1, wherein the nucleic acid vector is a cosmid.

8. The nucleic acid of claim 1, wherein the nucleic acid further encodes a second ribozyme under the control of a second **pol** III promoter.

9. The nucleic acid of claim 8, wherein the second **pol** III promoter is a Val promoter.

10. The nucleic acid of claim 1, wherein the ribozyme is selected from the group of ribozymes consisting of hammerhead ribozymes and hairpin ribozymes.

11. A cell transformed with the nucleic acid vector of claim 1.

12. A method of transforming a cell with a nucleic acid which encodes a ribozyme, comprising infecting the cell with the nucleic acid vector of claim 1.

13. The method of claim 12, wherein the ribozyme encoded by the nucleic acid specifically cleaves an HIV-1 nucleic acid.

14. The method of claim 12, wherein the cell is a stem cell.

L25 ANSWER 20 OF 21 USPATFULL on STN
97:117939 Methods and compositions for inhibiting production of replication competent virus.
Klump, Wolfgang M., Del Mar, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Chiron Corporation, United States (U.S. corporation)
US 5698446 19971216
APPLICATION: US 1994-305699 19940907 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods and compositions for inhibiting the production of replication competent virus. The invention comprises nucleic acid cassettes encoding a non-biologically active inhibitory molecule which are incorporated into packaging cells and recombinant vector constructs. Upon recombination between various vector construct contained within the producer cell, a biologically active molecule is produced which kills the cell, thereby inhibiting production of replication competent virus.
CLM What is claimed is:

1. A vector for directing the expression of a retroviral structural polypeptide, the vector comprising a promoter operably associated with a structural gene construct and a polyadenylation signal, the structural gene construct comprising a nucleic acid molecule coding for the retroviral structural polypeptide and a non-biologically active inhibitory molecule, wherein the vector inhibits production of replication competent retrovirus resulting from recombination events in retroviral packaging or producer cells.

2. The vector according to claim 1 wherein the retroviral structural polypeptide encoded by the structural gene construct is selected from the group consisting of retroviral env and **gag/pol**.

3. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a toxin selected from the group consisting of tetanus, ricin, and diphtheria toxin.

4. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a ribozyme.

5. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a prodrug activating enzyme.

6. The vector according to claim 1 wherein the nucleic acid molecule of the structural gene construct further comprises a splice site adjacent to be nucleic acid molecule.

7. A recombinant **retroviral vector** comprising; a) an LTR; b) a packaging signal; c) a tRNA binding site; d) a gene of interest; and e) a nucleic acid cassette comprising a nucleic acid molecule encoding a non-biologically active inhibitory molecule which results in a nucleic acid molecule encoding a biologically active inhibitory molecule upon recombination with the vector according to claim 1.

8. The packaging cell comprising the vector according to claim 1.

9. The vector according to claim 2 wherein the promoter is selected from the group consisting of an RSV promoter, adenovirus MLP, an SV40 promoter, and CMV MIE.

10. The vector according to claim 2 wherein the retroviral structural polypeptide is env derived from a retrovirus selected from the group consisting of **MoMLV**, 4070A, HTLV-I, HTLV-II, HIV, MPMV, SRV-I, HFV, MFV, SIV, GALV, BLV, FeLV, and FIV.

11. The vector according to claim 2 wherein the retroviral structural polypeptide is env selected from an amphotropic, polytropic or xenotropic retrovirus.

12. The vector according to claim 2 wherein **gag/pol** is derived from a **MoMLV** retrovirus.

13. The vector according to claim 11 wherein env is derived from a murine retrovirus.

14. The vector according to claim 5 wherein the non-biologically active inhibitory molecule is the prodrug activating enzyme HSVTK.

15. The recombinant vector according to claim 7 which further comprises a selectable marker.

16. The recombinant vector according to claim 7 wherein the nucleic acid molecule encoding the non-biologically active inhibitory molecule is contained in an LTR.

17. A producer cell comprising at least one vector encoding retroviral

gag/pol and env polypeptides and the recombinant retroviral **vector** according to claim 7.

18. The packaging cell according to claim 8 comprising a vector encoding a retroviral **gag/pol** polypeptide.

19. The packaging cell line according to claim 8 wherein said packaging cell is generated from D17 or HT1080 cells.

20. The packaging cell according to claim 18 further comprising another vector encoding a retroviral env polypeptide.

21. The retroviral packaging cell according to claim 18 further comprising another vector encoding a VSV G polypeptide.

22. A retroviral particle comprising a recombinant **retroviral vector** made by the producer cell according to claim 17.

23. A target cell transduced with the retroviral particle according to claim 22.

24. The target cell according to claim 23 that is an animal cell.

25. The target cell according to claim 24 wherein the animal cell is a human cell.

L25 ANSWER 21 OF 21 USPATFULL on STN
95:94825 Transduced fibroblasts.
Mulligan, Richard C., Cambridge, MA, United States
Wilson, James M., Waltham, MA, United States
Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
US 5460959 19951024
APPLICATION: US 1993-70646 19930601 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Fibroblasts transduced with genetic material encoding a polypeptide or protein of interest and, optionally, a selectable marker, as well as methods for making and using the transduced fibroblasts. Such fibroblasts are useful in delivering the encoded polypeptide or protein, such as an enzyme, a hormone or a drug, to an individual who has had a graft or implant of the transduced cells.
CLM What is claimed is:
1. Fibroblasts transduced by a recombinant retrovirus comprising incorporated genetic material, said transduced fibroblasts bound to a supporting matrix in vitro and having the property of being capable of expressing said incorporated genetic material in vivo.

2. Fibroblasts according to claim 1, wherein said incorporated genetic material encodes a polypeptide or protein.

3. Fibroblasts according to claim 1, wherein said incorporated genetic material comprises at least one gene encoding a selectable marker.

4. Fibroblasts according to claim 3, wherein said gene encoding a selectable marker encodes neomycin resistance.

5. Fibroblasts according to claim 1, wherein: (a) the genome of said recombinant retrovirus further comprises long terminal repeat sequences, a tRNA binding site, and a Psi packaging site derived from amphotropic **Moloney murine leukemia virus;** and (b) said recombinant retrovirus is amphotropic.

6. Fibroblasts according to claim 3, wherein: (a) the genome of said recombinant retrovirus further comprises long terminal repeat sequences,

a cell binding site, and a RSV packaging site derived from amphotropic **Moloney murine leukemia virus**; and (b) said recombinant retrovirus is amphotropic.

7. Fibroblasts according to claim 1, wherein the genome of said recombinant retrovirus further comprises a regulatable eukaryotic promoter.

8. Fibroblasts according to claim 3, wherein the genome of said recombinant retrovirus further comprises a regulatable eukaryotic promoter.

9. Fibroblasts according to claim 1, wherein said recombinant retrovirus is selected from the group consisting of **pRO**, pEM, and pIP.

10. Fibroblasts according to claim 3, wherein said recombinant retrovirus is selected from the group consisting of **pRO**, pEM, and pIP.

11. Fibroblasts according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein said supporting matrix comprises microcarrier beads.

12. A method of making transduced fibroblasts having the property of being capable of expressing incorporated genetic material in vivo, comprising the steps of: (a) binding fibroblasts to a supporting matrix; and (b) contacting said bound fibroblasts with a recombinant retrovirus comprising incorporated genetic material under conditions appropriate for retroviral infection.

13. The method of claim 12, wherein said incorporated genetic material encodes a polypeptide or protein.

14. The method of claim 12, wherein said incorporated genetic material comprises at least one gene encoding a selectable marker.

15. The method of claim 12, further comprising the step of culturing said fibroblasts under suitable growth conditions.

16. The method of claim 14, further comprising the step of culturing said fibroblasts under suitable growth conditions.

17. The method according to any one of claims 12, 13, 14, 15, or 16, wherein said supporting matrix comprises microcarrier beads.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2

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L9              2 S L8 NOT L6
                E KUHN R J/AU
L10            95 S E3
L11             9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12             8 S L11 NOT (L6 OR L9)
                E JEFFERS S A/AU
L13            33 S E2 OR E5
L14             2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15            10 S E3

     FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
                E SANDERS D A/IN
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                E FISCHBACH M A/IN
L17             1 S E3
                E KUHN R J/IN
L18             2 S E3
                E JEFFERS S A/IN
L19             3 S E3
                E NORTH C L/IN
L20             1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21         13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22          2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23           106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24            28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25            21 S L24 AND AY<2000

=> s l25 and (marker?/clm)
         18328 MARKER?/CLM
L26             12 L25 AND (MARKER?/CLM)

=> s l26 and (selectable/clm or detectable/clm)
         23433 SELECTABLE/CLM
         14687 DETECTABLE/CLM
L27             10 L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)

=> d l27,cbib,ab,clm,1-10

L27  ANSWER 1 OF 10  USPATFULL on STN
2003:142832 Pharmaceutical products comprising endothelial cell precursors.
    Isner, Jeffrey M., Weston, MA, United States
    Asahara, Takayuki, Arlington, MA, United States
    St. Elizabeth's Medical Center of Boston, Inc., Boston, MA, United States
    (U.S. corporation)
    US 6569428 B1 20030527
    APPLICATION: US 1999-228020  19990111 (9)                          <--
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB      Pharmaceutical products are provided comprising EC progenitors for use
        in methods for regulating angiogenesis, i.e., for enhancing or
        inhibiting blood vessel formation, in a selected patient and in some
        preferred embodiments for targeting an angiogenesis modulator to
        specific locations. For example, the EC progenitors can be used to
        enhance angiogenesis or to deliver an angiogenesis modulator, e.g.,
        anti- or pro-angiogenic agents, respectively to sites of pathologic or
        utilitarian angiogenesis. Additionally, in another embodiment, EC
        progenitors can be used to induce reendothelialization of an injured
        blood vessel, and thus reduce restenosis by indirectly inhibiting smooth
        muscle cell proliferation.
CLM     What is claimed is:
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1. A pharmaceutical product comprising a nucleic acid encoding an endothelial cell mitogen and endothelial cell (EC) progenitors, in a physiologically acceptable administrable form, wherein the EC progenitors are CD34+, Flk-1+, and tie-2+.

2. The pharmaceutical product of claim 1, wherein the nucleic acid comprises a vector.

3. The pharmaceutical product of claim 2, wherein the vector comprises sequence from a DNA or RNA virus.

4. The pharmaceutical product of claim 3, wherein the vector is a **retroviral vector**.

5. The pharmaceutical product of claim 4, wherein the **retroviral vector** comprises sequence from **moloney murine leukemia virus** or human immunodeficiency (HIV) virus.

6. The pharmaceutical product of claim 5, wherein the vector comprises human immunodeficiency virus (HIV) **gag** and **pol** genes.

7. The pharmaceutical product of claim 6, the product further comprising another vector comprising sequence from the human immunodeficiency (HIV) env gene.

8. The pharmaceutical product of claim 2, wherein the vector comprises sequence from a DNA virus.

9. The pharmaceutical product of claim 8, wherein the vector comprises sequence from at least one of pox virus, herpes virus, adenovirus, or adeno-associated virus.

10. The pharmaceutical product of claim 9, wherein the vector is replication defective.

11. The pharmaceutical product of claim 9, wherein the pox virus is orthopox or avipox.

12. The pharmaceutical product of claim 9, wherein the herpes virus is herpes simplex I virus (HSV).

13. The pharmaceutical product of claim 1, wherein the nucleic acid further comprises an operably linked promoter.

14. The pharmaceutical product of claim 13, wherein the promoter is a cytomegalovirus (CMV), Rous sarcoma virus (RSV), MMT promoter, or a native promoter.

15. The pharmaceutical product of claim 13, wherein the nucleic acid further comprises at least one enhancer.

16. The pharmaceutical product of claim 15, wherein the enhancer is a tat gene or tar element.

17. The pharmaceutical product of claim 2, wherein the vector comprises sequence encoding a **selectable marker**.

18. The pharmaceutical product of claim 1, wherein the encoded endothelial cell mitogen is sufficient to stimulate at least one of native EC cells to proliferate, migrate, remodel or form new sprouts from parental vessels.

19. The pharmaceutical product of claim 18, wherein the encoded endothelial cell mitogen comprises a secretory signal sequence.

20. The pharmaceutical product of claim 1, wherein the EC progenitors

21. The pharmaceutical product of claim 1, wherein the EC progenitors are detectably-labeled.

22. The pharmaceutical product of 21, wherein the detectably-labeled EC progenitors are radiolabeled.

23. The pharmaceutical product of claim 1, wherein the EC progenitors are obtained from human mononuclear cells, heterologous or autologous umbilical cord blood, or peripheral blood.

24. The pharmaceutical product of claim 23, wherein the EC progenitors are obtained from the leukocyte fraction of peripheral blood.

L27 ANSWER 2 OF 10 USPATFULL on STN
2001:208684 Cap-independent multicistronic **retroviral vectors.**
Adam, Mohammed A., Kirkland, Canada
Miller, A. Dusty, Seattle, WA, United States
Osborne, William Reginald Alfred, Seattle, WA, United States
Fred Hutchinson Cancer Research Center Board Regents of the University of Washington, Seattle, WA, United States (U.S. corporation)
US 6319707 B1 20011120
APPLICATION: US 1993-9338 19930126 (8) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB **Retroviral vectors** for producing coordinately expressed polycistronic mRNA in transfected host cells. A representative retroviral construct capable of forming a proviral genome in a host cell contains a first nucleotide coding sequence, a second nucleotide coding sequence, and a third nucleotide sequence capable of hybridizing under stringent conditions to a 5' nontranslated region (NTR) of a picornavirus RNA or its complementary RNA strand. The first, second, and third nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule containing transcripts of the first, second, and third nucleotide sequences. The transcript of the third nucleotide sequence in the messenger RNA molecule contains a nucleic acid capable of forming a regulatory stem-loop nucleic acid structure followed by at least one operable AUG start codon. The regulatory stem-loop nucleic acid structure is capable of operably binding a translation initiation complex in a host cell such that the transcripts of the first and second nucleotide sequences in the messenger RNA molecule are coordinately expressed in the host cell.
CLM What is claimed is:
1. A retroviral construct capable of forming a proviral genome in a host cell, said retroviral construct comprising: a first nucleotide sequence comprising a **MoMLV** LTR, a second nucleotide sequence comprising a first coding region, a third nucleotide sequence, comprising a picornavirus NTR, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a fourth nucleotide sequence comprising a second coding region, and a fifth nucleotide sequence comprising a poly-A tail, wherein said nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule comprising transcripts of the second, third, fourth, and fifth nucleotide sequences.

2. The retroviral construct of claim 1, wherein one of said first and second coding regions encodes a **detectable marker.**

3. The retroviral construct of claim 2, wherein the other of said first and second coding regions encodes a therapeutic gene product.

4. The retroviral construct of claim 1, comprising **MoMLV** retroviral elements for packaging and encapsidation of the retroviral RNA into a **retroviral vector** particle.

5. The retroviral construct of claim 1, wherein the transcript of the third nucleotide sequence in said messenger RNA molecule comprises a nucleic acid capable of forming a regulatory stem-loop nucleic acid structure followed by at least one operable AUG start codon.

6. The retroviral construct of claim 5, wherein the regulatory stem-loop nucleic acid structure is capable of operably binding a translation initiation complex in a host cell such that the transcripts of the second and fourth nucleotide sequences in said messenger RNA molecule are coordinately expressed in the host cell.

7. A packaging host cell transformed with the retroviral construct of claim 1, capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said proviral genome.

8. An infective **retroviral vector** particle encapsidated by the transformed packaging host cell of claim 7.

9. A producer host cell transduced with the **retroviral vector** particle of claim 8, the genome of said producer host cell comprising a first proviral genome corresponding to said retroviral construct in combination with a second proviral genome comprising a **gag** and **pol** gene and a third proviral genome comprising an env gene, said producer host cell being capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said first proviral genome.

10. An infective **retroviral vector** particle produced by the producer host cell of claim 9.

11. A target host cell transduced with the **retroviral vector** particle of claim 10, said target host cell containing said first proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

12. A producer host cell transformed with the retroviral construct of claim 1, the genome of said producer host cell comprising a first proviral genome corresponding to said retroviral construct in combination with a second proviral genome comprising a **gag** and **pol** gene and a third proviral genome comprising an env gene, said producer host cell being capable of encapsidating infective **retroviral vector** particles having a virion RNA complementary to said nucleotide sequences in said first proviral genome.

13. An infective **retroviral vector** particle produced by the producer host cell of claim 12.

14. A target host cell transduced with the **retroviral vector** particle of claim 13, said target host cell containing said first proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

15. A target host cell transformed with the retroviral construct of claim 1, said target host cell containing said proviral genome and being capable of expressing the gene products of said second and fourth nucleotide sequences.

16. A retroviral construct capable of forming a proviral genome in a host cell said retroviral construct comprising: a first nucleotide sequence comprising a **MoMLV** LTR a second nucleotide sequence comprising a first coding region, a third nucleotide sequence comprising a picornavirus NTR, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a fourth nucleotide sequence comprising a second coding region, a fifth nucleotide sequence

comprising a picornavirus IRES, wherein the picornavirus is selected from among encephalomyocarditis virus and poliovirus, a sixth nucleotide sequence comprising a third coding region, and a seventh nucleotide sequence comprising a poly-A tail, wherein said nucleotide sequences are operably linked such that transcription of the proviral genome gives rise to a messenger RNA molecule comprising transcripts of the said second, third, fourth, fifth, sixth, and seventh nucleotide sequences.

L27 ANSWER 3 OF 10 USPATFULL on STN
2001:55763 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Dull, Thomas J., San Francisco, CA, United States
Zsebo, Krisztina M., Woodside, CA, United States
Cooke, Keegan, Palo Alto, CA, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6218187 B1 20010417
APPLICATION: US 1999-266596 19990311 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.

CLM What is claimed is:

1. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

2. The method of claim 1, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

3. The method of claim 1, wherein said foreign gene encodes a chimeric T cell receptor.

4. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of; A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

5. The method of claim 4, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

6. The method of claim 4, wherein said foreign gene encodes a chimeric T cell receptor.

7. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding **gag** and **pol** proteins and a **selectable marker** wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population

of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

8. The method of claim 7, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

9. The method of claim 7, wherein said foreign gene encodes a chimeric T cell receptor.

10. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient transfection of a first population of mammalian cells stably transfected with at least one expression vector encoding **gag**, **pol** and env proteins and a **selectable marker** wherein the expression of **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

11. The method of claim 10, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

12. The method of claim 10, wherein said foreign gene encodes a chimeric T cell receptor.

13. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding adhesion molecules or antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian hematopoietic stem cells on said culture plates; and D) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

14. The method of claim 13, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

15. The method of claim 13, wherein said foreign gene encodes a chimeric T cell receptor.

16. The method of any one of claims 1, 4, 7, 10 or 13 wherein said adhesion molecules are selected from the group consisting of fibronectin and CS-1.

17. The method of any one of claims 1, 4, 7, 10 or 13 wherein said antibodies to adhesion molecules are selected from the group consisting of antibodies to VLA-4, VLA-5, CD29, CD11a, CD11b and CD44.

18. A method to transduce mammalian T and B lymphocytes with **retroviral vectors** produced by transient transfection comprising the

steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

19. The method of claim 18, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

20. The method of claim 18, wherein said foreign gene encodes a chimeric T cell receptor.

21. The method of claim 18, further comprising infecting a second population of mammalian target cells with the supernatant from said mammalian cells of claim 18 to transduce said target cells with a foreign gene.

22. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging the helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding the **gag** and **pol** proteins of ectropic MMLV or GALV and a second retroviral helper sequence comprises a cDNA encoding the envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

23. The method of claim 22, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

24. The method of claim 22, wherein said foreign gene encodes a chimeric T cell receptor.

25. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding the **gag** and **pol** proteins and a **selectable marker** wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

26. The method of claim 25, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

27. The method of claim 25, wherein said foreign gene encodes a chimeric T cell receptor.

28. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient transfection of a first population of mammalian cells stably transfected with at least one expression vector encoding the **gag**, **pol** and env proteins and a **selectable marker** wherein the expression of the **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

29. The method of claim 28, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

30. The method of claim 28, wherein said foreign gene encodes a chimeric T cell receptor.

31. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian T or B lymphocytes on said culture plates; and D) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

32. The method of claim 31, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

33. The method of claim 31, wherein said foreign gene encodes a chimeric T cell receptor.

34. The method of any one of claims 18, 22, 25, 28 or 31 wherein said antibodies to adhesion molecules is selected from the group consisting of antibodies to LFA-1, CD-2, CD40 and gp39.

35. The method of claims 1, 4, 7, 10, 13, 18, 22, 25, 28 or 31, wherein the first population of mammalian cells comprises a human cell.

36. An improved method to efficiently transduce mammalian cells with a retroviral supernatant, comprising the steps of: i) growing said population of mammalian cells on culture plates; and ii) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying a foreign gene with said population of mammalian cells, to transduce said population of mammalian cells with said foreign gene, whereby target cells efficiently transduced with said foreign gene are obtained, wherein the improvement comprises adding antibodies to adhesion molecules present on said population of mammalian cells to culture plates.

37. The target cell of claim 36, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

38. The target cell of claim 37, wherein said foreign gene encodes a chimeric T cell receptor.

39. The target cell of claim 38, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

40. The method of claim 3, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

41. The method of claim 6, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane

domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

42. The method of claim 9, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

43. The method of claim 12, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

44. The method of claim 15, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

45. The method of claim 20, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

46. The method of claim 24, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

47. The method of claim 27, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

48. The method of claim 30, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

49. The method of claim 33, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

50. The method of claim 35, wherein said human cell is a 293 cell.

51. The method of claim 21, wherein said target cells are lymphocytes or hematopoietic stem cells.

L27 ANSWER 4 OF 10 USPATFULL on STN
2000:47084 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States
Roberts, Margo R., Charlottesville, VA, United States
Dull, Thomas L., San Francisco, CA, United States
Zsebo, Krisztina M., Cupertino, CA, United States
Qin, Lu, Lutherville, MD, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6051427 20000418
APPLICATION: US 1995-517488 19950821 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.
CLM What is claimed is:
1. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-imcompetent retroviral genome encoding in trans all virion proteins required and for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said helper DNA sequence codes for ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV) **gag** and **pol**, and an envelope protein or chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

2. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus

and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV), and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

3. The stable packaging cell line comprising helper sequences encoding **gag** and **pol** proteins designated 35.32.

4. A human embryonic kidney cell stably transfected with an expression vector encoding **gag** and **pol** proteins and a **selectable marker**, wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent.

5. The human embryonic kidney cell of claim 4 wherein the **gag** and **pol** are derived from **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV).

6. The human embryonic kidney cell of claim 4 wherein said cell is either 293 or tsa54.

7. A human embryonic kidney cell stably transfected with two expression vectors wherein the first expression vector encodes **gag** and **pol** proteins and the second expression vector encodes an envelope protein.

8. The human embryonic kidney cell of claim 7 wherein said cell is either 293 or tsa54 and said gal and **pol** proteins and derived from **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (HIV).

9. The human embryonic kidney cell of claim 7 or 10 wherein said envelope protein is derived from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

10. The human embryonic kidney cell of claim 9, wherein said envelope protein is comprised of sequences from two or more of said viruses.

11. A stable packaging cell line comprising helper sequences encoding **gag**, **pol** and envelope proteins designated 37S2.8.

12. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking the both the psi function sequence responsible for packaging helper genome and the 3'LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said foreign enhancer is the RSV enhancer

and promoter.

13. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic **Moloney murine leukemia virus** (MMLV), gibbon ape leukemia virus (GALV) or immunodeficiency virus (HIV) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, HIV, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II; and wherein said foreign enhancer is the RSV enhancer and promoter.

L27 ANSWER 5 OF 10 USPATFULL on STN
2000:9750 Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant.
Rigg, Richard J., Mountain View, CA, United States
Chen, Jingyi, Fremont, CA, United States
Dando, Jonathan S., Milan, Italy
Plavec, Ivan, Menlo Park, CA, United States
Forestell, Sean P., Menlo Park, CA, United States
Bohnlein, Ernst, Los Altos, CA, United States
SyStemix, Inc., Palo Alto, CA, United States (U.S. corporation)
US 6017761 20000125
WO 9721825 19970619
APPLICATION: US 1997-817452 19970415 (8) <--
WO 1996-US20777 19961213 19970415 PCT 371 date 19970415 PCT 102(e) date <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** as well as the recombinant packaging cell obtained by the method. Also provided is a method of producing recombinant retroviral particles obtained by introducing into the packaging cells obtained according to the methods disclosed herein, a recombinant **retroviral vector** and propagating the resulting producer cells under conditions favorable for the production and secretion of **retroviral vector** supernatant. The retroviral supernatant produced by these methods also is claimed herein. This invention further provides a method for screening **retroviral vector** supernatant for high transduction efficiency and methods for producing **retroviral vector** supernatant for transducing cells with high efficiency in gene therapy applications.

CLM What is claimed is:
1. A method for obtaining a recombinant retroviral packaging cell comprising: a. isolating a retroviral nucleic acid sequence encoding a minimal **gag-pol** open reading frame (ORF), said nucleic acid sequence having no flanking sequences of the **gag-pol** ORF, and inserting said nucleic acid sequence into a first expression plasmid; b. isolating a retroviral nucleic acid sequence encoding a minimal env ORF, said nucleic acid sequence having no flanking sequences of the env ORF, and

inserting said nucleic acid sequence into a second expression plasmid; c. obtaining a eukaryotic cell free of an endogenous nucleic acid sequence which encodes the **gag-pol** ORF or the env ORF and which is derived from the retrovirus from which the minimal **gag-pol** ORF or env ORF is isolated; and d. introducing the first and second expression plasmids into the eukaryotic cell and expressing the nucleic acids encoding the minimal **gag-pol** ORF and env ORF to produce **Gag, Pol** and Env proteins, thereby producing the recombinant retroviral packaging cell.

2. The method of claim 1, wherein the retrovirus is a murine leukemia virus.

3. The method of claim 1, wherein the cell is a non-murine cell.

4. The method of claim 3, wherein the non-murine cell is a primate cell.

5. The method of claim 4, wherein the primate cell is a human cell.

6. The method of claim 3, wherein the non-murine cell is selected from the group consisting of Vero, HT-1080, D17 MRC-5, TE671, human embryonic kidney, and HeLa cells.

7. The method of claim 6, wherein the human embryonic kidney cells are human 293 cells (ATCC CRL 1573).

8. The method of claim 1, wherein the **gag-pol** ORF is a **Moloney murine leukemia virus gag-pol** gene.

9. The method of claim 1, wherein the env ORF is a murine leukemia virus env gene.

10. The method of claim 1, wherein at least one of the expression plasmids further comprises a **selectable** or **detectable marker** gene.

11. The method of claim 1, wherein the screening is done by ELISA.

12. The method of claim 11, wherein in the ELISA, Env is detected using a primary antibody from hybridoma 83A25 followed by antiserum 79S-834, enzyme-conjugated antispecies antibody and enzyme substrate; and **Gag** is detected separately using a primary antibody from hybridoma R187 followed by antiserum 77S-227, enzyme-conjugated antispecies antibody and enzyme substrate.

13. The method of claim 8 wherein the **gag-pol** gene is expressed from the MMLV-LTR promoter.

14. The method of claim 8, wherein the **gag-pol** gene is expressed from the CMV-IE promoter or the RSV-LTR promoter.

15. The method of claim 1 wherein the **gag-pol** ORF and env ORF are isolated from the same retrovirus.

16. The method of claim 1, wherein the **gag-pol** ORF and env ORF are isolated from different retroviruses.

17. The method of claim 1, wherein the first and second expression plasmids are introduced into the eukaryotic cell in separate and sequential steps.

18. The method of claim 1, further comprising screening the cell of step (d) for retroviral **Gag, Pol** and Env production.

19. The recombinant retroviral packaging cell obtained by the method of claim 1.

20. The retroviral packaging cell of claim 19, wherein the cell produces an amphotropic Env.

21. The retroviral packaging cell of claim 19, wherein the cell produces a xenotropic Env.

22. The retroviral packaging cell of claim 19, wherein the cell produces a chimeric amphotropic/xenotropic Env.

23. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is a non-murine cell.

24. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is derived from human 293 having ATCC Accession No. CRL 1573.

25. The recombinant retroviral packaging cell of claim 19 wherein the packaging cell is a primate cell.

26. A method of producing a **retroviral vector** producer cell which comprises transducing the cells of claim 19 or 23 with a retroviral-based vector and subsequently propagating the cell under conditions favorable for the production and secretion of **retroviral vector** supernatant.

27. The method of claim 26, further comprising screening the producer cell for the ability to produce a vector supernatant having high transduction efficiency, comprising measuring the ability of the vector supernatant to transduce a target cell population with a transduction efficiency greater than that achieved with a vector supernatant produced from murine PA317-based cells.

28. The method of claim 27, wherein the target cell population is human 293 cells (ATCC CRL 1573).

29. The method of claim 26, wherein the retroviral-based vector used to transduce the cells was produced in human cells.

30. The **retroviral vector** producer cell produced by the method of claim 26.

31. A method of increasing the gene transduction efficiency of a cell, comprising transducing the cell with a **retroviral vector** supernatant produced from the culture of at least one **retroviral vector** producer cell of claim 30, wherein the transduction efficiency is increased over that achieved with a vector supernatant produced from murine PA317-based cells.

32. The method of claim 31, wherein the **retroviral vector** producer cell is derived from a packaging cell selected from group consisting of ProPak-A.6 (ATCC Accession No. CRL 12006), ProPak-A.52 (ATCC Accession No. CRL-12479) or ProPak-X.36 (ATCC Accession No. CRL 12007).

33. The method of claim 31 wherein the **retroviral vector** supernatant is produced from the co-culture of a first and a second complementary **retroviral vector** producer cell without replication competent retrovirus generation.

34. The method of claim 30 wherein the first **retroviral vector** producer cell is derived from an amphotropic packaging cell and the second vector producer cell is derived from a xenotropic packaging cell.

35. The method of claim 34 wherein the amphotropic packaging cell is ProPak-A.6 (ATCC Accesssion No. CRL 12006) or ProPak-A.52 (ATCC Accession No. CRL-12479), and the xenotropic packaging cell is ProPak-X.36 (ATCC Accesssion No. CRL 12007).

36. The method of claim 34 wherein both the amphotropic and xenotropic packaging cells are produced from human 293 cells (ATCC CRL 1573).

37. The method of claim 31 wherein the **retroviral vector** supernatant is produced from a stable **retroviral vector** producer cell culture.

38. The method of claim 31 wherein the transduced cell is a primary human hematopoietic cell.

39. The method of claim 31 wherein the transduced cell is a human hematopoietic stem cell.

40. The method of claim 38 wherein the hematopoietic cell is a CD34+ Thy1+ cell from mobilized peripheral blood or a CD4+ PBL.

41. The retroviral packaging cell of claim 19, capable of packaging **retroviral vector** sequences to form a **retroviral vector** producer cell that does not generate RCR after continuous culture for up to at least 12 weeks and that produces a recombinant, transducing **retroviral vector** particle, the **retroviral vector** particle characterized by: a. being resistant to human complement; and b. having a high transduction efficiency.

42. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak-A.6 and has ATCC Accesssion No. CRL 12006.

43. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak A.52 having ATCC Accession No. CRL-12479.

44. The retroviral packaging cell line of claim 41 wherein the cell line is designated ProPak-X.36 and has ATCC Accession No.CRL 12007.

45. An expression plasmid for expressing **gag-pol**, comprising a **gag-pol** open reading frame from the start codon to the stop codon with no flanking sequences of the open reading frame.

46. An expression plasmid for expressing env, comprising an env open reading frame from the start codon to the stop codon with no flanking sequences of the open reading frame.

L27 ANSWER 6 OF 10 USPATFULL on STN
1999:128827 Pantropic **retroviral vectors** for gene transfer in mollusks.
Burns, Jane C., La Jolla, CA, United States
Chen, Thomas T., Storrs, CT, United States
Ther Regents of The University of California, Oakland, CA, United States (U.S. corporation)The University of Conneticut, Farmington, CT, United States (U.S. corporation)
US 5969211 19991019
APPLICATION: US 1997-844530 19970418 (8) <--
PRIORITY: US 1996-16253P 19960419 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method for introducing foreign nucleic acid sequences into marine mollusks. A pantropic **retroviral vector** containing a foreign gene sequence is introduced into fertilized mollusk embryos by electroporation. The gene sequence becomes integrated into the host DNA and encodes a functional protein product. This method has implications in the introduction of disease-resistance and growth-accelerating genes into mollusks.
CLM What is claimed is:
1. A method for introducing exogenous DNA into a germline of a marine mollusk, comprising the steps of: inserting said exogenous DNA into a plasmid comprising a promoter that is active in mollusks and retroviral long terminal repeats (LTRs), wherein said exogenous DNA is operably

linked to said promoter, thereby generating a recombinant vector, forming a pseudotyped retrovirus containing VSV G protein and RNA corresponding to said recombinant vector; and infecting a mollusk embryo with said pseudotyped retrovirus so as to insert said exogenous DNA into the germline of said mollusk.

2. The method of claim 1, wherein said marine mollusk is a clam.

3. The method of claim 1, wherein said marine mollusk is selected from the group consisting of oyster, mussel, scallop and abalone.

4. The method of claim 1, wherein said retroviral long terminal repeats are **Moloney murine leukemia virus** long terminal repeats.

5. The method of claim 1, wherein said infecting step is performed by electroporation.

6. The method of claim 1, wherein said infecting step is performed by dechorionation or microinjection.

7. The method of claim 1, wherein the inserting step comprises inserting said exogenous DNA into a first plasmid containing retroviral long terminal repeats, a gene encoding a **selectable marker**, and said promoter is operably linked to said exogenous DNA.

8. The method of claim 7, wherein the forming step comprises: transfecting a packaging cell line with said first plasmid, wherein said cell line assembles vector particles containing an RNA copy of said recombinant vector; infecting a producer cell line with said vector particles, said producer cell line containing retroviral **gag** and **pol**; transfecting said vector particle-containing producer cell line with a second plasmid containing a promoter operably linked to VSV G protein, whereby pseudotyped **retroviral vectors** containing the VSV-G protein are secreted by said producer cell line.

9. The method of claim 7, wherein said first plasmid is pLSRNL or pGeo4.8.

10. The method of claim 7, wherein said promoter is the Rous sarcoma virus promoter.

11. The method of claim 7, wherein said **selectable marker** is neomycin phosphotransferase.

12. The method of claim 8, wherein said packaging cell line is PA317.

13. The method of claim 8, wherein said producer cell line is 293 cells.

14. The method of claim 8, wherein said second plasmid is pHCMV-G.

15. The method of claim 8, wherein said promoter of said second plasmid is cytomegalovirus promoter.

L27 ANSWER 7 OF 10 USPATFULL on STN
1999:65191 Method for obtaining retroviral packaging cell lines producing high transducing efficiency retroviral supernatant.
Rigg, Richard J., Mountain View, CA, United States
Chen, Jingyi, Fremont, CA, United States
Dando, Jonathan S., Sunnyvale, CA, United States
Plavec, Ivan, Menlo Park, CA, United States
Forestell, Sean P., Menlo Park, CA, United States
Bohnlein, Ernst, Los Altos, CA, United States
SyStemix, Inc., Palo Alto, CA, United States (U.S. corporation)
US 5910434 19990608
APPLICATION: US 1995-572959 19951215 (8) <--

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention provides a method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** and the recombinant packaging cell obtained by the method. Also provided is a method of producing recombinant retroviral particles obtained by introducing into the packaging cells obtained according to the methods disclosed herein, a recombinant **retroviral vector** and propagating the resulting producer cells under conditions favorable for the production and secretion of **retroviral vector** supernatant. The retroviral supernatants produced by these methods also is claimed herein. This invention further provides a method for screening **retroviral vector** supernatant for high transduction efficiency and methods for producing **retroviral vector** supernatant for transducing cells with high efficiency in gene therapy applications.

CLM What is claimed is:

1. A method for obtaining a recombinant retroviral packaging cell capable of producing **retroviral vectors** comprising: a. selecting a retrovirus; b. obtaining a eukaryotic cell free of endogenous retroviral nucleic acid of the same type as the retrovirus of step (a); c. preparing a minimal **gag-pol** open reading frame (ORF) insert from the retrovirus wherein the ORF contains no flanking sequences of the **gag-pol** gene; d. inserting the minimal **gag-pol** ORF prepared from step (c) into an appropriate expression plasmid, wherein the **gag-pol** ORF is operatively linked to a heterologous promoter having no overlap with the **retroviral vector**; e. preparing a minimal env open reading frame (ORF) insert from the retrovirus wherein the ORF contains no flanking sequences of the env gene; f. inserting the minimal env ORF prepared from step (e) into an appropriate expression plasmid, wherein the env ORF is operatively linked to a heterologous promoter having no overlap with the **retroviral vector**; g. inserting the expression plasmids of steps (d) and (f) into the cell of step (b); h. propagating the cell obtained from step (g) under conditions favorable for expression of the minimal retroviral **gag-pol** and env ORF; and i. screening a cell for retroviral **Gag**, **Pol** and Env production by the cell of step (h); thereby obtaining the retroviral packaging cell capable of packaging recombinant **retroviral vector** sequences to produce recombinant, transducing retrovirus.

2. The method of claim 1, wherein the retrovirus is a **Moloney murine leukemia virus**.

3. The method of claim 1, wherein the cell is a non-murine cell.

4. The method of claim 3, wherein the non-murine cell is a primate cell.

5. The method of claim 4, wherein the primate cell is a human cell.

6. The method of claim 3, wherein the non-murine cell is selected from the group consisting of Vero, HT-1080, D17 MRC-5, FS-4, TEG71, human embryonic kidney (293), and HeLa.

7. The method of claim 6, wherein the human embryonic kidney cells are 293 cells (ATCC CRL 1573).

8. The method of claim 1, wherein the **gag-pol** ORF is a **moloney murine leukemia virus gag-pol** gene.

9. The method of claim 8, wherein the **gag-pol** gene is expressed from the CMV-IE promoter or the RSV-LTR promoter.

10. The method of claim 1, wherein the env ORF is a **moloney murine leukemia virus** env gene.

11. The method of claim 1, wherein the plasmid of steps (d) or (f) comprises a **selectable** or **detectable marker** gene.

12. The method of claim 1, wherein the **gag-pol** ORF and the env ORF expression plasmids of step (g) are amplified in bacterial host cells prior to inserting into the eukaryotic cell of step (b), wherein the bacterial host cells are propagated at a temperature range from about 28° C. to about 32° C.

13. The method of claim 12, wherein the bacterial host cells are propagated at about 30° C.

14. The method of claim 1, wherein in step (i), **Gag**, **Pol** and Env production is screened by a sandwich ELISA assay.

15. The method of claim 14, wherein Env is detected using a primary antibody from hybridoma 83A25 followed by antiserum 79S-834, enzyme-conjugated antispecies antibody and enzyme substrate; and **Gag** is detected separately using a primary antibody from hybridoma R187 followed by antiserum 77S-227, enzyme-conjugated antispecies antibody and enzyme substrate.

16. The recombinant retroviral packaging cell obtained by the method of claim 1.

17. The recombinant retroviral packaging cell of claim 16, wherein the cell produces an amphotropic env.

18. The recombinant retroviral retroviral packaging cell of claim 16, wherein the cell produces a xenotropic env.

19. A retroviral packaging cell line designated ProPak-A.6 having ATCC Accession No. CRL 12006.

20. A method of producing a retroviral producer cell which comprises transducing the cells of claim 16 with a retroviral-based vector and subsequently propagating the cells under conditions favorable for the production and secretion of **retroviral vector** supernatant.

21. The method of claim 20, further comprising screening the producer cell for the ability to produce a vector supernatant having high transduction efficiency, comprising measuring the ability of the vector supernatant to transduce a target cell population with a transduction efficiency greater than that achieved with a vector supernatant produced from murine PA317-based cells.

22. The method of claim 21, wherein the target cell population is human 293 cells.

23. The retroviral producer cell produced by the method of claim 20.

L27 ANSWER 8 OF 10 USPATFULL on STN
1998:122246 VSV G pseusdotyped **retroviral vectors**.
Yee, Jiing-Kuan, Del Mar, CA, United States
Emi, Nobuhiko, Nagoya, CA, United States
Friedmann, Theodore, La Jolla, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Barber, Jack R., San Diego, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)Chiron Viagene, Inc., Emeryville, CA, United States (U.S. corporation)
US 5817491 19981006
APPLICATION: US 1994-361839 19941222 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB An enveloped vector particle contains gag and pol proteins from a retrovirus, a nucleic acid sequence and an envelope that includes VSV G

envelope glycoprotein. The vector particle can be used to introduce nucleic acids into cells.

CLM What is claimed is:

1. An enveloped vector particle comprising: **gag** and **pol** proteins from a retrovirus; a nucleic acid sequence associated with said vector particle; an envelope having vesicular stomatitis virus (VSV) G envelope glycoprotein therein.

2. The vector particle of claim 1, wherein said **gag** and **pol** proteins are from the same species of retrovirus.

3. The vector particle of claim 2, wherein the nucleic acid sequence encodes a gene having an origin other than from said retrovirus, said gene being expressible into polypeptide.

4. The vector particle of claim 3, in which said polypeptide is a **selectable marker**.

5. The vector particle of claim 4, wherein the **selectable marker** is expressed by a neomycin resistance gene.

6. The vector particle of claim 3, wherein said nucleic acid sequence additionally comprises a promoter which can direct the transcription of said gene.

7. The vector particle of claim 2, wherein said **gag** and **pol** proteins are from **MoMLV**.

8. A method of introducing foreign nucleic acid into a cell, said method comprising infecting said cell with the enveloped vector particle of claim 1.

9. The method of claim 8, wherein said **gag** and **pol** proteins are from the same species of retrovirus.

10. The method of claim 9, wherein said cell is outside the normal host range of the retrovirus.

11. A method according to claim 9, wherein the retrovirus has a host range not including human cells or a subset of human cells.

12. A method according to claim 9, wherein the retrovirus is **MoMLV**.

13. A cell line that stably expresses or carries **gag/pol**, transfected or transduced with a nucleic acid molecule encoding a **retroviral vector** and a nucleic acid molecule encoding a VSV G protein, the cell line being capable of producing functional vector particles.

14. The cell line of claim 13, wherein the cell line is transiently transfected.

15. A 293 cell stably expressing **gag/pol**.

16. The cell of claim 15, wherein the cell is a 293 2-3 cell.

17. A method of making a VSV G pseudotyped virus, comprising: introducing into a cell line that stably expresses **gag/pol** a VSV G protein expression vector and a **retroviral vector** construct, the resulting cell line being capable of producing vector particles comprising VSV G protein.

18. The method of claim 17, wherein the **retroviral vector** construct is introduced prior to the VSV G protein expression vector.

19. The method of claim 18, wherein the method further comprises

selecting for cells carrying the **retroviral vector** construct prior to introducing the VSV G protein expression vector.

20. A method of making a VSV G pseudotyped virus, comprising: (a) cotransfecting or sequentially transfecting a cell with a vector that directs the expression of **gag/pol** and a **retroviral vector** construct; and (b) introducing a VSV G protein expression vector into the cotransfected or sequentially transfected cell, the resulting cell being capable of producing vector particles comprising VSV G protein.

21. The method of claim 20, wherein the cell is cotransfected, and the method includes selecting for transfected cells prior to introducing a VSV G protein expression vector.

22. The method of claim 20, wherein the cell is sequentially transfected, and the method include selecting for cells expressing either **gag/pol** or the **retroviral vector** construct.

L27 ANSWER 9 OF 10 USPATFULL on STN
97:117939 Methods and compositions for inhibiting production of replication competent virus.
Klump, Wolfgang M., Del Mar, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Chiron Corporation, United States (U.S. corporation)
US 5698446 19971216
APPLICATION: US 1994-305699 19940907 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods and compositions for inhibiting the production of replication competent virus. The invention comprises nucleic acid cassettes encoding a non-biologically active inhibitory molecule which are incorporated into packaging cells and recombinant vector constructs. Upon recombination between various vector construct contained within the producer cell, a biologically active molecule is produced which kills the cell, thereby inhibiting production of replication competent virus.

CLM What is claimed is:
1. A vector for directing the expression of a retroviral structural polypeptide, the vector comprising a promoter operably associated with a structural gene construct and a polyadenylation signal, the structural gene construct comprising a nucleic acid molecule coding for the retroviral structural polypeptide and a non-biologically active inhibitory molecule, wherein the vector inhibits production of replication competent retrovirus resulting from recombination events in retroviral packaging or producer cells.

2. The vector according to claim 1 wherein the retroviral structural polypeptide encoded by the structural gene construct is selected from the group consisting of retroviral env and **gag/pol**.

3. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a toxin selected from the group consisting of tetanus, ricin, and diphtheria toxin.

4. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a ribozyme.

5. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a prodrug activating enzyme.

6. The vector according to claim 1 wherein the nucleic acid molecule of the structural gene construct further comprises a splice site adjacent to be nucleic acid molecule.

7. A recombinant **retroviral vector** comprising; a) an LTR; b) a

packaging signal, b, a tRNA binding site, c, a gene of interest, and d, a nucleic acid cassette comprising a nucleic acid molecule encoding a non-biologically active inhibitory molecule which results in a nucleic acid molecule encoding a biologically active inhibitory molecule upon recombination with the vector according to claim 1.

8. The packaging cell comprising the vector according to claim 1.

9. The vector according to claim 2 wherein the promoter is selected from the group consisting of an RSV promoter, adenovirus MLP, an SV40 promoter, and CMV MIE.

10. The vector according to claim 2 wherein the retroviral structural polypeptide is env derived from a retrovirus selected from the group consisting of **MoMLV**, 4070A, HTLV-I, HTLV-II, HIV, MPMV, SRV-I, HFV, MFV, SIV, GALV, BLV, FeLV, and FIV.

11. The vector according to claim 2 wherein the retroviral structural polypeptide is env selected from an amphotropic, polytropic or xenotropic retrovirus.

12. The vector according to claim 2 wherein **gag/pol** is derived from a **MoMLV** retrovirus.

13. The vector according to claim 11 wherein env is derived from a murine retrovirus.

14. The vector according to claim 5 wherein the non-biologically active inhibitory molecule is the prodrug activating enzyme HSVTK.

15. The recombinant vector according to claim 7 which further comprises a **selectable marker**.

16. The recombinant vector according to claim 7 wherein the nucleic acid molecule encoding the non-biologically active inhibitory molecule is contained in an LTR.

17. A producer cell comprising at least one vector encoding retroviral **gag/pol** and env polypeptides and the recombinant **retroviral vector** according to claim 7.

18. The packaging cell according to claim 8 comprising a vector encoding a retroviral **gag/pol** polypeptide.

19. The packaging cell line according to claim 8 wherein said packaging cell is generated from D17 or HT1080 cells.

20. The packaging cell according to claim 18 further comprising another vector encoding a retroviral env polypeptide.

21. The retroviral packaging cell according to claim 18 further comprising another vector encoding a VSV G polypeptide.

22. A retroviral particle comprising a recombinant **retroviral vector** made by the producer cell according to claim 17.

23. A target cell transduced with the retroviral particle according to claim 22.

24. The target cell according to claim 23 that is an animal cell.

25. The target cell according to claim 24 wherein the animal cell is a human cell.

Genetically transduced fibroblasts.
Mulligan, Richard C., Cambridge, MA, United States
Wilson, James M., Waltham, MA, United States
Whitehead Institute for Biomedical Research, Cambridge, MA, United States (U.S. corporation)
US 5460959 19951024
APPLICATION: US 1993-70646 19930601 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Fibroblasts transduced with genetic material encoding a polypeptide or protein of interest and, optionally, a selectable marker, as well as methods for making and using the transduced fibroblasts. Such fibroblasts are useful in delivering the encoded polypeptide or protein, such as an enzyme, a hormone or a drug, to an individual who has had a graft or implant of the transduced cells.

CLM What is claimed is:

1. Fibroblasts transduced by a recombinant retrovirus comprising incorporated genetic material, said transduced fibroblasts bound to a supporting matrix in vitro and having the property of being capable of expressing said incorporated genetic material in vivo.

2. Fibroblasts according to claim 1, wherein said incorporated genetic material encodes a polypeptide or protein.

3. Fibroblasts according to claim 1, wherein said incorporated genetic material comprises at least one gene encoding a **selectable marker**.

4. Fibroblasts according to claim 3, wherein said gene encoding a **selectable marker** encodes neomycin resistance.

5. Fibroblasts according to claim 1, wherein: (a) the genome of said recombinant retrovirus further comprises long terminal repeat sequences, a tRNA binding site, and a Psi packaging site derived from amphotropic **Moloney murine leukemia virus**; and (b) said recombinant retrovirus is amphotropic.

6. Fibroblasts according to claim 3, wherein: (a) the genome of said recombinant retrovirus further comprises long terminal repeat sequences, a tRNA binding site, and a Psi packaging site derived from amphotropic **Moloney murine leukemia virus**; and (b) said recombinant retrovirus is amphotropic.

7. Fibroblasts according to claim 1, wherein the genome of said recombinant retrovirus further comprises a regulatable eukaryotic promoter.

8. Fibroblasts according to claim 3, wherein the genome of said recombinant retrovirus further comprises a regulatable eukaryotic promoter.

9. Fibroblasts according to claim 1, wherein said recombinant retrovirus is selected from the group consisting of **pRO**, pEM, and pIP.

10. Fibroblasts according to claim 3, wherein said recombinant retrovirus is selected from the group consisting of **pRO**, pEM, and pIP.

11. Fibroblasts according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, wherein said supporting matrix comprises microcarrier beads.

12. A method of making transduced fibroblasts having the property of being capable of expressing incorporated genetic material in vivo, comprising the steps of: (a) binding fibroblasts to a supporting matrix; and (b) contacting said bound fibroblasts with a recombinant retrovirus comprising incorporated genetic material under conditions appropriate for retroviral infection.

13. The method of claim 12, wherein said incorporated genetic material encodes a polypeptide or protein.

14. The method of claim 12, wherein said incorporated genetic material comprises at least one gene encoding a **selectable marker.**

15. The method of claim 12, further comprising the step of culturing said fibroblasts under suitable growth conditions.

16. The method of claim 14, further comprising the step of culturing said fibroblasts under suitable growth conditions.

17. The method according to any one of claims 12, 13, 14, 15, or 16, wherein said supporting matrix comprises microcarrier beads.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21 13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT

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L22          [illegible] S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23          106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24           28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25           21 S L24 AND AY<2000
L26           12 S L25 AND (MARKER?/CLM)
L27           10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)

=> s l21 and (lentivir?/clm or FIV/clm or HIV/clm or SIV/clm or BIV/clm or EIAV/clm)
            372 LENTIVIR?/CLM
            134 FIV/CLM
           4382 HIV/CLM
            144 SIV/CLM
             65 BIV/CLM
             34 EIAV/CLM
L28          689 L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BIV/C
                 LM OR EIAV/CLM)

=> s l28 and (lentivir? expression vector/clm)
            372 LENTIVIR?/CLM
          34993 EXPRESSION/CLM
          44687 VECTOR/CLM
              5 LENTIVIR? EXPRESSION VECTOR/CLM
                  ((LENTIVIR?(W)EXPRESSION(W)VECTOR)/CLM)
L29           5 L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)

=> d l29,cbib,1-5

L29  ANSWER 1 OF 5  USPATFULL on STN
2004:52619 Transgenic organism.
    Radcliffe, Philippa, Oxford, UNITED KINGDOM
    Mitrophanous, Kyriacos, Oxford, UNITED KINGDOM
    Themis, Michael, London, UNITED KINGDOM
    Oxford BioMedica (UK) Limited (non-U.S. corporation)
    US 2004040052 A1 20040226
    APPLICATION: US 2003-421947  A1  20030424 (10)
    PRIORITY: GB 2001-30797  20011221
    GB 2002-1140  20020118
    GB 2002-11409  20020517
    DOCUMENT TYPE: Utility; APPLICATION.

L29  ANSWER 2 OF 5  USPATFULL on STN
2003:194507 Methods of using randomized libraries of zinc finger proteins for
    the identification of gene function.
    Case, Casey C., San Mateo, CA, UNITED STATES
    Liu, Qiang, Foster City, CA, UNITED STATES
    Rebar, Edward J., El Cerrito, CA, UNITED STATES
    Wolffe, Alan P., Orinda, CA, UNITED STATES
    Sangamo BioSciences, Inc. (U.S. corporation)
    US 2003134318 A1 20030717
    APPLICATION: US 2003-337216  A1  20030106 (10)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L29  ANSWER 3 OF 5  USPATFULL on STN
2003:175213 Transgenic organism.
    Radcliffe, Philippa, Oxford, UNITED KINGDOM
    Mitrophanous, Kyriacos, Oxford, UNITED KINGDOM
    Themis, Michael, London, UNITED KINGDOM
    Oxford BioMedica (UK) Limited (non-U.S. corporation)
    US 2003121062 A1 20030626
    APPLICATION: US 2002-82122  A1  20020226 (10)
    PRIORITY: GB 2001-30797  20011221
    GB 2002-1140  20020118
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

L29 ANSWER 4 OF 5 USPATFULL on STN
2002:265815 Methods of using randomized libraries of zinc finger proteins for the identification of gene function.
Case, Casey C., San Mateo, CA, UNITED STATES
Liu, Qiang, Foster City, CA, UNITED STATES
Rebar, Edward J., El Cerrito, CA, UNITED STATES
Wolffe, Alan P., Orinda, CA, UNITED STATES
US 2002146691 A1 20021010
APPLICATION: US 2000-731558 A1 20001206 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L29 ANSWER 5 OF 5 USPATFULL on STN
2002:198257 Methods for therapy of neurodegenerative disease of the brain.
Tuszynski, Mark H., La Jolla, CA, UNITED STATES
Regents of the University of California (U.S. corporation)
US 2002106350 A1 20020808
APPLICATION: US 2001-32952 A1 20011026 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

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     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21        13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22         2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23          106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24           28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25           21 S L24 AND AY<2000
L26           12 S L25 AND (MARKER?/CLM)
L27           10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28          689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29            5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)

=> s l28 and ay<2000
       2989870 AY<2000
L30         283 L28 AND AY<2000

=> s l30 and (gag/clm or pro/clm or pol/clm)
            973 GAG/CLM
           7363 PRO/CLM
            547 POL/CLM
L31          80 L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)

=> s l31 and (marker?/clm)
          18328 MARKER?/CLM
L32          16 L31 AND (MARKER?/CLM)

=> d l32,cbib,ab,clm,1-16

L32  ANSWER 1 OF 16  USPATFULL on STN
2003:279110 Retrovirus and viral vectors.
    Lauermann, Vit, Baltimore, MD, United States
    Rubicon Laboratory, Inc., Baltimore, MD, United States (U.S. corporation)
    US 6635472 B1 20031021
    APPLICATION: US 1998-134360  19980814 (9)                          <--
    PRIORITY: US 1997-55864P  19970815 (60)
    US 1998-91734P  19980706 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     This invention relates to the fields of genetic engineering, virus
       replication and gene transfer. More specifically, this invention relates
       to polynucleotide construct, recombinant virus, transposon, and their
       vectors, wherein an ori derived from a DNA virus capable of replicating
       in vertebrate cells is inserted into the retrovirus, allowing the
       retrovirus following the reverse transcription to efficiently replicate
       as extrachromosomal or episomal DNA without the necessity of integration
       into the host cell chromosome. Additionally, this invention relates to
       polynucleotide construct, recombinant virus, transposon, and their
       vectors replicating episomally without aid of an ori and related
       elements. Also, this invention encompasses preventive, therapeutic, and
       diagnostic applications employing said constructs, viruses and vectors.
CLM    What is claimed is:
       1. A polynucleotide construct comprising retroviral sequence encoding at
       least one LTR, polypurine tract and packaging signal of a retroviral
       genome of an episomally replicating retrovirus, which is able to
       replicate without requirement of integration, said retroviral sequences
       further comprising one or more mutations that disable the integration of
       said construct into host chromosomal DNA, said construct further having
       the capacity to replicate via reverse transcription, provided that any
       reverse transcription product obtained from such reverse transcription
       is also disabled from integrating into host chromosomal DNA, said
       retroviral sequence further comprising a heterologous sequence encoding
       a gene product of interest.

       2. The polynucleotide construct of claim 1 in which said retroviral
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sequence further comprise the 5' and 3' LTRs.

3. The polynucicotide construct of claim 1 in which said retroviral genome is selected from the group consisting of **HIV**, HTLV, MLV, AMV, ALV, BLV, SSV, RSV, CAEV, **SIV**, ERV, EAIV and **FIV**.

4. The polynucleotide construct of claim 1 in which said retroviral sequence further comprise an origin of DNA replication.

5. The polynucleotide construct of claim 4 in which said origin of DNA replication is one found in a DNA virus.

6. The polynucleotide construct of claim 5 in which said DNA virus is selected from the group consisting of papova viruses or herpes viruses.

7. The polynucleotide construct of claim 1 in which said one or more mutations are within an inverted repeat of a LTR or an integrase.

8. The polynucleotide construct of claim 1 which further comprise a capsid, polymerase, protease, integrase, envelope, auxiliary region, or combination of same.

9. The polynucleotide construct of claim 1 in which said heterologous sequence is a foreign gene.

10. The polynucleotide construct of claim 1 in which said heterologous sequence is a vertebrate gene.

11. The polynucleotide construct of claim 9 in which said foreign gene is either defective or absent from a host cell.

12. The polynucleotide construct of claim 1 in combination with retroviral genes carried by one or more helper constructs, wherein said combination encodes integration defective infectious virions.

13. A composition comprising retroviral sequence encoding all the genetic elements necessary for the production of an immunogenic virion, including one or more LTRs, said genetic elements including one or more mutations that disable the integration of viral DNA into host chromosomal DNA, such that any DNA molecules arising from a reverse transcription step involving an RNA of said immunogenic virion are able to exist episomally within host vertebrate cells, said virion further being able to replicate without requirement of integration, said retroviral sequence further comprising a heterologous sequence encoding a gene product of interest.

14. The retroviral sequence of claim 13 in which said episomal existence provides an immunogenic virion that can stimulate an immune system of a vertebrate host.

15. The retroviral sequence of claim 13 in which said immunogenic virion is a retrovirus.

16. The composition of claim 15 in which said retrovirus is selected from the group consisting of MLV, AMV, ALV, BLV, SSV, RSV, CAEV, **HIV**, HTLV, **SIV**, ERV, EAIV, or **FIV**.

17. The composition of claim 13 which used in cancer cells.

18. The retroviral sequence of claim 13 which is able to exist episomally within selected cells of a vertebrate host.

19. The heterologous sequence of claim 13 which comprises nucleotide sequences encoding a cytokine or chemokine.

20. The heterologous sequence of claim 13 which comprises a gene

encoding a protein that converts a **pro** drug into a cytotoxic agent.

21. The heterologous sequence of claim 13 which comprises one or more tumor **markers** expressed in selected cells of a host into which said composition has been introduced.

22. The heterologous sequence of claim 21 which said one or more tumor **markers** are selected from the group consisting of a suppressor gene or an oncogene.

23. The heterologous sequence of claim 22 in which said suppressor gene is selected from a group consisting of p53, p73, p51, p40, or ket gene.

24. The heterologous sequence of claim 22 in which said oncogene is selected from a group consisting of c-myc, c-jun, c-fos, c-rel, c-qin, c-neu, c-src, c-abl, c-lck, c-mil/raf, c-ras, c-sis, or c-fps.

L32 ANSWER 2 OF 16 USPATFULL on STN
2003:142832 Pharmaceutical products comprising endothelial cell precursors.
Isner, Jeffrey M., Weston, MA, United States
Asahara, Takayuki, Arlington, MA, United States
St. Elizabeth's Medical Center of Boston, Inc., Boston, MA, United States (U.S. corporation)
US 6569428 B1 20030527
APPLICATION: US 1999-228020 19990111 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Pharmaceutical products are provided comprising EC progenitors for use in methods for regulating angiogenesis, i.e., for enhancing or inhibiting blood vessel formation, in a selected patient and in some preferred embodiments for targeting an angiogenesis modulator to specific locations. For example, the EC progenitors can be used to enhance angiogenesis or to deliver an angiogenesis modulator, e.g., anti- or pro-angiogenic agents, respectively to sites of pathologic or utilitarian angiogenesis. Additionally, in another embodiment, EC progenitors can be used to induce reendothelialization of an injured blood vessel, and thus reduce restenosis by indirectly inhibiting smooth muscle cell proliferation.

CLM What is claimed is:

1. A pharmaceutical product comprising a nucleic acid encoding an endothelial cell mitogen and endothelial cell (EC) progenitors, in a physiologically acceptable administrable form, wherein the EC progenitors are CD34+, Flk-1+, and tie-2+.

2. The pharmaceutical product of claim 1, wherein the nucleic acid comprises a vector.

3. The pharmaceutical product of claim 2, wherein the vector comprises sequence from a DNA or RNA virus.

4. The pharmaceutical product of claim 3, wherein the vector is a **retroviral vector**.

5. The pharmaceutical product of claim 4, wherein the **retroviral vector** comprises sequence from moloney murine leukemia virus or human immunodeficiency (**HIV**) virus.

6. The pharmaceutical product of claim 5, wherein the vector comprises human immunodeficiency virus (**HIV**) **gag** and **pol** genes.

7. The pharmaceutical product of claim 6, the product further comprising another vector comprising sequence from the human immunodeficiency (**HIV**) env gene.

8. The pharmaceutical product of claim 2, wherein the vector comprises

sequence from a DNA virus.

9. The pharmaceutical product of claim 8, wherein the vector comprises sequence from at least one of pox virus, herpes virus, adenovirus, or adeno-associated virus.

10. The pharmaceutical product of claim 9, wherein the vector is replication defective.

11. The pharmaceutical product of claim 9, wherein the pox virus is orthopox or avipox.

12. The pharmaceutical product of claim 9, wherein the herpes virus is herpes simplex I virus (HSV).

13. The pharmaceutical product of claim 1, wherein the nucleic acid further comprises an operably linked promoter.

14. The pharmaceutical product of claim 13, wherein the promoter is a cytomegalovirus (CMV), Rous sarcoma virus (RSV), MMT promoter, or a native promoter.

15. The pharmaceutical product of claim 13, wherein the nucleic acid further comprises at least one enhancer.

16. The pharmaceutical product of claim 15, wherein the enhancer is a tat gene or tar element.

17. The pharmaceutical product of claim 2, wherein the vector comprises sequence encoding a selectable **marker**.

18. The pharmaceutical product of claim 1, wherein the encoded endothelial cell mitogen is sufficient to stimulate at least one of native EC cells to proliferate, migrate, remodel or form new sprouts from parental vessels.

19. The pharmaceutical product of claim 18, wherein the encoded endothelial cell mitogen comprises a secretory signal sequence.

20. The pharmaceutical product of claim 1, wherein the EC progenitors are angioblasts.

21. The pharmaceutical product of claim 1, wherein the EC progenitors are detectably-labeled.

22. The pharmaceutical product of 21, wherein the detectably-labeled EC progenitors are radiolabeled.

23. The pharmaceutical product of claim 1, wherein the EC progenitors are obtained from human mononuclear cells, heterologous or autologous umbilical cord blood, or peripheral blood.

24. The pharmaceutical product of claim 23, wherein the EC progenitors are obtained from the leukocyte fraction of peripheral blood.

L32 ANSWER 3 OF 16 USPATFULL on STN
2003:115732 Fusion protein delivery system and uses thereof.
Kappes, John C., Birmingham, AL, United States
Wu, Xiaoyun, Birmingham, AL, United States
UAB Research Foundation, Birmingham, AL, United States (U.S. corporation)
US 6555342 B1 20030429
APPLICATION: US 1999-460548 19991214 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a composition of matter, comprising: DNA

encoding a viral Vpr protein fused to DNA encoding a protein. In another embodiment of the present invention, there is provided a composition of matter, comprising: DNA encoding a viral Vpr protein fused to DNA encoding a protein. The present invention further provides DNA, vectors and methods for expressing a lentiviral pol gene in trans, independent of the lentiviral gag-pol. A gene transduction element is optionally delivered to a lentiviral vector according to the present invention. Also provided are various methods of delivering a virus inhibitory molecule to a target in an animal. Further provided is a pharmaceutical composition.

CLM What is claimed is:

1. A transretroviral vector system comprising: at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first nucleic acid segment capable of expression in a mammalian cell, said functional portion of said first **Gag** protein capable of providing for the incorporation of said fusion protein into a viral particle.

2. The transretroviral vector system of claim 1 further comprising at least a second nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second nucleic acid segment capable of expression in said mammalian cell, said functional portion of said second **Gag** polypeptide and said protease are capable of supporting the formation of an infectious viral particle, and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide.

3. The transretroviral vector system according to claim 2 further comprising at least a third nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said third nucleic acid segment provided on a different or the same nucleic acid strand as said first and said second nucleic acid segments.

4. The transretroviral vector system according to claim 3 further comprising at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell.

5. The transretroviral vector system according to claim 4 wherein said fourth nucleic acid segment further comprises a promoter sequence active in the target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth nucleic acid segment into the target cell.

6. The transretroviral vector system according to any of claims 2, 3, or 4 wherein said functional portions of said first and said second **Gag** protein and said protease are from a retrovirus.

7. The transretroviral vector of claim 5 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

8. The transretroviral vector system according to claim 6 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

9. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in

frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first nucleic acid segment capable of expression in a mammalian cell, said functional portion of said first **Gag** protein capable of providing for the incorporation of said fusion protein into a viral particle; and, (b) at least a second nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first nucleic acid segment, said second nucleic acid segment capable of expression in said mammalian cell, said functional portion of said second **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle, and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide.

10. The transretroviral vector system according to claim 9 further comprising at least a third nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said third nucleic acid segment provided on a different or the same nucleic acid strand as said first and said second nucleic acid segments.

11. The transretroviral system according to claim 10 further comprising at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell.

12. The transretroviral system according to claim 11 wherein said fourth nucleic acid segment further comprises a promoter sequence active in the target cell and operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth segment into the target cell.

13. The transretroviral system according to any of claims 9, 10, or 11 wherein said functional portions of said first and said second **Gag** protein and said protease polypeptide are from a retrovirus.

14. The transretroviral system of claim 12 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

15. The transretroviral vector system according to claim 13 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

16. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first segment capable of expression in a mammalian cell, said functional portion of said first **Gag** protein capable of providing for the incorporation of said fusion protein into a viral particle; (b) at least a second nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second nucleic acid segment capable of expression in said mammalian cell, said functional portion of said second **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle and wherein said second nucleic acid segment does not encode a functional Reverse

transcriptase polypeptide or a functional integrase polypeptide, and (c) at least a third nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said third nucleic acid segment provided on a different or the same nucleic acid strand as said first and said second nucleic acid segments.

17. The transretroviral vector system according to claim 16 further comprising at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell.

18. The transretroviral vector system according to claim 17 wherein said fourth nucleic acid segment further comprises a promoter sequence active in the target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth segment into the target cell.

19. The transretroviral vector system according to any of claims 16 or 17 wherein said functional portions of said first and said second **Gag** protein and said protease polypeptide are from a retrovirus.

20. The transretroviral vector of claim 18 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

21. The transretroviral vector system according to claim 19 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

22. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first nucleic acid segment capable of expression in a mammalian cell, said functional portion of said first **Gag** protein capable of providing for the incorporation of said fusion protein into a viral particle; (b) at least a second nucleic acid segment comprising a nucleotide sequence encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second nucleic acid segment capable of expression in said mammalian cell, said functional portion of said second **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle, and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; (c) at least a third nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different or same nucleic acid strand as said first and said second nucleic acid segments; and (d) at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell.

23. The transretroviral vector system according to claim 22 wherein said fourth nucleic acid segment further comprises a promoter sequence active in the target cell operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth nucleic acid segment into the target cell.

24. The transretroviral vector system according to claim 22 wherein said functional portion of said first or said second **Gag** protein and said

protease polypeptide are from a lentivirus.

25. The transretroviral vector system of claim 23 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

26. The transretroviral vector system according to claim 24 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

27. The transretroviral vector system according to any of claims 1, 9, 16, or 22 wherein said first nucleic acid segment further comprises a nucleic acid sequence comprising the Rev Responsive Element.

28. The transretroviral vector system of claim 22, wherein said nucleotide sequence of interest encodes a virus inhibitory polypeptide and said vector in association with a pharmaceutically acceptable carrier.

29. The transretroviral vector system according to any of claims 1, 11, 17, or 22 wherein at least one of said first, second, third, or fourth nucleic acid segment further comprises a gene encoding a **marker** protein selected from the group consisting of β-gal, fluorescence proteins, and luciferase.

30. The transretroviral vector system of claims 1, 11, 17, or 22, wherein said nucleotide sequence of interest encodes a polypeptide.

31. The transretroviral vector system of claims 5, 12, 18, or 23, wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS, WPRE and sequences capable of stabilizing messenger RNAs and increasing the titer of the viral particle.

32. The transretroviral vector system according to any of claims 1, 11, or 17 further comprising promoters operatively linked to at least one of said first, said second, said third or said fourth nucleic acid segments, said promoters selected from the group consisting of **HIV** promoters, non-**HIV** promoters, constitutive promoters, and inducible promoters.

33. The transretroviral vector system according to any of claims 1, 11, 17, or 22 further comprising a poly A signal operatively lined to at least one of said first, second, third or fourth nucleic acid segments, said poly A signal selected from the group consisting of non **HIV** poly A, SV40 poly A, and non-**lentiviral** poly A.

34. The transretroviral vector system according to claim 30, wherein said protein is selected from the group consisting of a viral inhibitory protein and a therapeutic protein.

35. The transretroviral vector system according to claim 30 wherein said nucleotide sequence of interest encodes a protein selected from the group consisting of neomycin, hygromycin, and puromycin.

36. A method for generating a transretroviral vector comprising: (a) providing at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first segment capable of expression in a mammalian cell, said functional portion of said **Gag** polypeptide capable of providing for the incorporation of said fusion protein into a viral particle; (b) providing at least a second nucleic acid segment comprising a nucleic acid sequence encoding at least a functional

portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second nucleic acid segment capable of expression in a mammalian cell, said functional portion of said second **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle, and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; (c) providing at least a third nucleic acid segment comprising nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different or same nucleic acid strand as said first or said second nucleic acid segments; (d) contacting said nucleic acid segments of (a), (b), and (c) with the mammalian cell, said mammalian cell becoming transfected with said first, said second, and said third nucleic acid segment; and, (e) producing viral particles from said mammalian cell, said viral particles containing said fusion protein.

37. The method according to claim 36 further comprising providing at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell.

38. The method according to claim 37 wherein said fourth nucleic acid segment further comprises a promoter active in said target cell and operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth nucleic acid segment into the target cell.

39. The method according to claim 38 wherein said nucleic acid sequence for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

40. A method for generating a transretroviral vector comprising: (a) providing at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said segment capable of expression in a mammalian cell, said functional portion of said first **Gag** polypeptide capable of providing for the incorporation of said fusion protein into a viral particle; (b) providing at least a second nucleic acid segment encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second nucleic acid segment capable of expression in the mammalian cell, said functional portion of said second **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional integrase polypeptide; (c) providing at least a third nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence for packaging, reverse transcription and integration of said third nucleic acid segment into the genome of a target cell; (d) contacting said nucleic acids of (a), (b), and (c) with the mammalian cell, said mammalian cell becoming transfected with said first, said second, and said third nucleic acid segment; and, (e) producing viral particles from said mammalian cell, said particles containing said fusion protein.

41. The method according to claim 40 further comprising providing at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different

of same nucleic acid strand as said first, said second, or said third nucleic acid segment.

42. The method according to claim 40 wherein said third segment further comprises a promoter sequence active in said target cell and operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said third segment into said target cell.

43. The method according to claim 42 wherein said nucleic acid sequence for facilitating transduction is selected from the group consisting of PPT-CTS and WPRE.

44. The method of claims 38 or 42, wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS, WPRE and sequences capable of stabilizing messenger RNAs and increasing he titer of the viral particle.

45. A method for generating a transretroviral vector comprising: (a) providing at least a first nucleic acid segment encoding at least one fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a reverse transcriptase polypeptide having reverse transcriptase activity fused in frame to a functional portion of an integrase polypeptide having integrase activity, said first nucleic acid segment capable of expression in a mammalian cell, said functional portion of said first **Gag** polypeptide capable of providing for the incorporation of said fusion protein into a viral particle; (b) providing at least a second nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a second **Gag** polypeptide and a functional portion of a protease polypeptide, said second nucleic acid segment provided on the same or a different nucleic acid strand than said first segment, said second segment capable of expression in said mammalian cell, said functional portion of said second **Gag** polypeptide and a protease polypeptide are capable of supporting the formation of an infectious viral particle and wherein said second nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; (c) providing at least a third nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different or same nucleic acid strand as said first or said second nucleic acid segments; (d) providing at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell; (e) contacting said nucleic acid segment of (a), (b), (c) and (d) with the mammalian cell, said mammalian cell becoming tranfected with said nucleic acids; and, (f) producing viral particles from said mammalian cell, said particles containing said fusion protein.

46. The method according to claim 45 wherein said fourth nucleic acid segment further comprises a promoter sequence active in said target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth segment into the target cell.

47. The method according to claim 36, 40, or 45 wherein said first nucleic acid segment further comprises a Rev Responsive Element (RRE).

48. The method of claim 45 wherein said nucleotide sequence of interest encodes a viral inhibitory protein and said vector is in association with a pharmaceutically acceptable carrier.

49. The method of claims 37 or 45 wherein at least one of said first, said second, said third, or said fourth nucleic acid segment further comprise a nucleic acid sequence encoding a **marker** protein, said

marker protein selected from the group consisting of β-gal, fluorescence proteins, and luciferase.

50. The method of claims 37 or 45 wherein said first, said second, said third, and said fourth nucleic acid segment further comprise a promoter active in said mammalian cell operatively linked to at least one of said first, said second, said third, and said fourth nucleic acid segments, said promoter selected from the group consisting of **HIV** promoters, non-**HIV** promoters, constitutive promoters, and inducible promoters.

51. The method of claims 37 or 45 wherein at least one of said first, said second, said third, or said fourth nucleic acid segment further comprise providing a poly A signal operatively linked to at least one of said first, second, third, or fourth nucleic acid segments, said poly A signal selected from the group consisting of non **HIV** poly A, SV40 poly A, and non-**lentiviral** poly A.

52. The method of claims 37 or 45, wherein said nucleotide sequence of interest encodes a polypeptide.

53. The method according to claim 46 wherein said nucleic acid sequence for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

54. The method of claim 52 wherein said nucleotide sequence of interest encodes a viral inhibitory protein or a therapeutic protein.

55. The method of claim 52 wherein said nucleotide sequence of interest encodes at least one drug resistant protein selected from the group consisting of neomycin, hygromycin, and puromycin.

56. A transretroviral vector system comprising: at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein is capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand.

57. The transretroviral vector system of claim 56 further comprising: at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle and said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide.

58. The transretroviral vector system according to claim 57 further comprising at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said fourth nucleic acid segment provided on a different or the same nucleic acid strand as said first, said second, or said third nucleic acid segments.

59. The transretroviral vector system according to claim 58 further comprising at least a fifth nucleic acid segment comprising a nucleic

acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a target cell.

60. The transretroviral vector system according to claim 59 wherein said fifth nucleic acid segment further comprises a promoter active in the target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth nucleic acid segment into the target cell.

61. The transretroviral vector of claim 60 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

62. The transretroviral vector system according to any of claims 57, 58, and, 59 wherein said functional portions of said first, said second, said third **Gag** polypeptide and said protease polypeptide are from a retrovirus.

63. The transretroviral vector system according to claim 62 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

64. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein are capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide.

65. The transretroviral vector system according to claim 64 further comprising at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said fourth nucleic acid segment provided on a different or the same nucleic acid strand as said first, said second, or said third nucleic acid segments.

66. The transretroviral vector system according to claim 65 further comprising at least a fifth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a target cell.

67. The transretroviral vector system according to claim 66 wherein said fifth nucleic acid segment further comprises a promoter active in the target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth segment into the target cell.

68. The transretroviral vector system according to any of claims 64. 65, or 66 wherein said functional portions of said first, said second, and said third **Gag** protein and said protease polypeptide are from a retrovirus.

69. The transretroviral viral vector of claim 67 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

70. The transretroviral vector system according to claim 68 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

71. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; and, (c) at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said fourth nucleic acid segment provided on a different or the same nucleic acid strand as said first, said second, said third, or said fourth nucleic acid segment.

72. The transretroviral vector system according to claim 71 further comprising at least a fifth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a host cell.

73. The transretroviral vector system according to any of claims 71 or 72 wherein said functional portions of said first, said second, said third **Gag** protein and said protease polypeptide are from a retrovirus.

74. The transretroviral vector system according to claim 72 wherein said fifth nucleic acid segment further comprises a promoter active in the target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth segment into the target cell.

75. The transretroviral vector of claim 74 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

76. The transretroviral vector system according to claim 73 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**,

77. A transretroviral vector system comprising: (a) at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; and, (c) at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said fourth nucleic acid segment provided on a different or the same nucleic acid strand as said first, said second, said third, or said fourth nucleic acid segment; and, (d) at least a fifth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a target cell.

78. The transretroviral vector system according to claim 77 wherein said fifth nucleic acid segment further comprises a promoter active in the target cell operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth nucleic acid segment into the target cell.

79. The transretroviral vector system according to claim 77 wherein said functional portion of said first, said second or said third **Gag** protein and said protease polypeptide are from a retrovirus.

80. The transretroviral vector system according to any of claims 56, 64, 71, or 77 wherein said first and said second nucleic acid segment further comprises a nucleic acid sequence comprising a Rev Responsive Element (RRE).

81. The transretroviral vector system according to any of claims 59, 66, 72, or 77 wherein at least one of said first, second, third, fourth or fifth nucleic acid segment further comprises a nucleotide sequence encoding a **marker** protein selected from the group consisting of β-gal, fluorescence proteins, and luciferase.

82. The transretroviral vector system according to claim 79 wherein said retrovirus is selected from the group consisting of **HIV**, **SIV**, **EIAV**, **BIV**, **FIV**, and MLV.

83. The transretroviral vector of claim 78 wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

84. The transretroviral vector system according to any of claims 59, 66,

said first, said second, said third, said fourth, or said fifth nucleic acid segments, said promoters selected from the group consisting of **HIV** promoters, non-**HIV** promoters, constitutive promoters, and inducible promoters.

85. The transretroviral vector system according to any of claims 59, 66, 72, or 77 further comprising a poly A signal operatively linked to at least one of said first, second, third, fourth or fifth nucleic acid segments, said poly A signal selected from the group consisting of non **HIV** poly A, SV40 poly A, and non-**lentiviral** poly A.

86. The transretroviral vector system of claims 59, 66, 72, or 77, wherein said nucleotide sequence of interest encodes a polypeptide.

87. The transretroviral vector system of claims 60, 67, 74, or 78, wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS, WPRE and sequences capable of stabilizing messenger RNAs and increasing the titer of the viral particle.

88. The transretroviral vector system according to claim 86 wherein said nucleotide sequence of interest encodes a protein which confers resistance to bacteria and is selected from the group consisting of neomycin, hygromycin, and puromycin.

89. The transretroviral vector system according to claim 86, wherein said nucleotide sequence of interest encodes a polypeptide selected from the group consisting of a viral inhibitory protein and a therapeutic protein.

90. A method for generating a transretroviral vector comprising: (a) providing at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, providing at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) providing at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; and, (c) providing at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said fourth nucleic acid segment provided on a different or the same nucleic acid strand as said first, said second, said third, or said fourth nucleic acid segment; and, (d) contacting said nucleic acid segments of (a), (b), and (c) with the mammalian cell, said mammalian cell becoming transfected with said first, said second, said third, and said fourth nucleic acid segment; and, (e) providing viral particles from said mammalian cell, said viral particles containing said first and said second fusion protein.

91. The method according to claim 90 further comprising providing at least a fifth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a target cell.

92. The method according to claim 91 wherein said fifth nucleic acid segment further comprises a promoter active in said target cell and operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth nucleic acid segment into the target cell.

93. The method according to claim 92 wherein said nucleic acid sequence for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

94. A method for generating a transretroviral vector system comprising: (a) providing at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, providing at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) providing at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; (c) providing at least a fourth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fourth nucleic acid segment into the genome of a target cell; (d) contacting said nucleic acids of (a), (b), and (c) with the mammalian cell, said mammalian cell becoming transfected with said first, said second, said third and said fourth nucleic acid segment; and, (e) providing viral particles from said mammalian cell, said particles containing said first and said second fusion protein.

95. The method according to claim 94 further comprising providing at least a fifth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different or same nucleic acid strand as said first, said second, said third, or said forth nucleic acid segment.

96. The method according to claim 94 wherein said fourth segment further comprises a promoter active in said target cell and operably linked to said nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fourth segment into said target cell.

97. The method according to claim 96 wherein said nucleic acid sequence for facilitating transduction are selected from the group consisting of

98. A method for generating a transretroviral vector comprising: (a) providing at least a first nucleic acid segment encoding a first fusion protein comprising at least a functional portion of a first truncated **Gag** protein fused in frame to a functional portion of a Reverse Transcriptase polypeptide having reverse transcriptase activity; and, providing at least a second nucleic acid segment encoding a second fusion protein comprising at least a functional portion of a second truncated **Gag** protein fused in frame to a functional portion of an Integrase polypeptide having integrase activity; wherein said first and said second nucleic acid segment capable of expression in a mammalian cell; said functional portion of said first and said second **Gag** protein capable of providing for the incorporation of said first and said second fusion protein into a viral particle; and, said first and said second nucleic acid sequences provided on the same or different nucleic acid strand; (b) providing at least a third nucleic acid segment comprising a nucleic acid sequence encoding at least a functional portion of a third **Gag** polypeptide and a functional portion of a protease polypeptide, wherein, said third nucleic acid segment provided on the same or a different nucleic acid strand than said first or said second nucleic acid segment; said third nucleic acid segment capable of expression in said mammalian cell; said functional portion of said third **Gag** polypeptide and said protease polypeptide are capable of supporting the formation of an infectious viral particle; and, wherein said third nucleic acid segment does not encode a functional Reverse Transcriptase polypeptide or a functional Integrase polypeptide; (c) providing at least a fourth nucleic acid segment comprising a nucleic acid sequence encoding an envelope polypeptide, said segment provided on a different or same nucleic acid strand as said first, said second, or said third nucleic acid segments; (d) providing at least a fifth nucleic acid segment comprising a nucleic acid sequence of interest and at least one cis acting nucleic acid sequence functioning in packaging, reverse transcription and integration of said fifth nucleic acid segment into the genome of a target cell; (e) contacting said nucleic acids of (a), (b), (c), and (d) with the mammalian cell, said mammalian cell becoming transfected with said first, said second, said third, said fourth and said fifth nucleic acid segment; and, (f) providing viral particles from said mammalian cell, said particles containing said first and said second fusion protein.

99. The method according to claim 98 wherein said fifth nucleic acid segment further comprises a promoter active in said target cell and operably linked to the nucleotide sequence of interest and at least one nucleic acid sequence for facilitating transduction of said fifth segment into the target cell.

100. The method according to claims 90, 94, 98 wherein at least said first or said second nucleic acid segment further comprises a Rev Responsive Element (RRE).

101. The method of claims 91 or 98 wherein at least said first, said second, said third, said fourth or said fifth nucleic acid segment further comprise a nucleic acid sequence encoding a **marker** protein, said **marker** protein selected from the group consisting of β-gal, fluorescence proteins, and luciferase.

102. The method of claims 91 or 98 wherein at least said first, said second, said third, said fourth, or said fifth nucleic acid segment further comprise a promoter active in said mammalian cell operatively linked to at least one of said first, said second, said third, said fourth, or said fifth nucleic acid segments, said promoter selected from the group consisting of **HIV** promoters, non-**HIV** promoters, constitutive promoters, and inducible promoters.

103. The method of claims 91 or 98 wherein at least said first, said

second, said third, said fourth or said fifth nucleic acid segments further comprise providing a poly A signal operatively linked to at least one of said first, second, third, fourth or fifth nucleic acid segments, said poly A signal selected from the group consisting of non **HIV** poly A, SV40 poly A, and non-**lentiviral** poly A.

104. The method of claims 91 or 98, wherein said nucleotide sequence of interest encodes a polypeptide.

105. The method of claims 92, 96, or 99, wherein said nucleic acid sequences for facilitating transduction are selected from the group consisting of PPT-CTS, WPRE, and sequences capable of stabilizing messenger RNAs and increasing the titer of the viral particle.

106. The method according to claim 99 wherein said nucleic acid sequence for facilitating transduction are selected from the group consisting of PPT-CTS and WPRE.

107. The method of claim 104 wherein said nucleotide sequence of interest encodes a viral inhibitory protein or a therapeutic protein.

108. The method of claim 104 wherein said nucleotide sequence of interest encodes at least one drug resistant protein selected from the group consisting of neomycin, hygromycin, and puromycin.

L32 ANSWER 4 OF 16 USPATFULL on STN
2002:217078 Retroviral hybrid vectors pseudotyped with LCMV.
Von Laer, Meike-Dorothee, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Beyer, Winfried, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Heinrich-Pette-Institut, Hamburg, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
US 6440730 B1 20020827
APPLICATION: US 1999-309572 19990511 (9) <--
PRIORITY: DE 1998-19856463 19981126
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates in general to the pseudotyping of retroviruses with lymphocytic choriomeningitis virus. In particular, the invention relates to pseudotyping in MLV packaging cells which are optionally env-deleted, or in packaging cells derived from lentiviruses. Preferably, pseudotyping takes place by infection with LCMV or a preferably env-deleted mutant, or by transfection with an expression plasmid containing the gp gene of LCMV or a part thereof and optionally, in addition, the np, 1 and/or the z gene of LCMV. The invention also relates to the use of such pseudotypes for the infection of cells, particularly the use in gene therapy.
CLM What is claimed is:
1. A retroviral packaging cell, which contains **gag** and **pol** genes of a **retroviral vector** and a nucleic acid sequence coding for the glycoproteins GP-1 and GP-2 of lymphocytic choriomeningitis virus (LCMV), wherein the **gag** and **pol** genes and said glycoproteins are expressed in said retroviral packaging cell.

2. The retroviral packaging cell according to claim 1, which also contains at least one gene from the group consisting of an env gene of a **retroviral vector**, regulatory retroviral genes, the gene np of LCMV coding for the nucleoprotein, the gene I of LCMV coding for RNA polymerase and the gene z of LCMV coding for a protein with an unknown function.

3. The retroviral packaging cell according to claim 1, wherein the **retroviral vector** is a MLV-related **retroviral vector** or a **lentiviral** vector.

4. The retroviral packaging cell according to claim 1, wherein the

retroviral vector is derived from MLV, MPV, SIV or FIV.

5. The retroviral packaging cell according to claim 1 further comprising a recombinant **retroviral vector** containing one or more transgenes selected from the group consisting of **marker** genes and genes encoding therapeutic proteins, wherein the retroviral packaging cell produces recombinant retroviral virions.

6. The retroviral packaging cell according to claim 5, wherein the **marker** genes are neo, lacZ or EGFP.

7. The retroviral packaging cell according to claim 1, further comprising a recombinant **retroviral vector** containing a ribozyme, an antisense sequence or a transdominant-negative acting gene, wherein the retroviral packaging cell produces recombinant retroviral virions.

8. The retroviral packaging cell according to claim 1 which is infected with lymphocytic choriomeningitis virus (LCMV), wherein said retroviral packaging cell further comprises one or more foreign genes.

9. The retroviral packaging cell according to claim 8, wherein the **retroviral vector** is derived from a MLV which does not express an Env protein, and wherein LCMV is the defective mutant L(ARM).

10. The retroviral packaging cell according to claim 1, which is obtained by a transfection of a packaging cell with an expression plasmid which comprises the nucleic acid sequence coding for glycoproteins GP-1 and GP-2 of LCMV.

11. The retroviral packaging cell according to claim 1, which is obtained by a transfection of a packaging cell with an expression plasmid which comprises the nucleic acid sequence coding for glycoproteins GP-1 and GP-2 of LCMV, and at least one of the np gene of LCMV, the/gene of LCMV or the z gene of LCMV.

12. The retroviral packaging cell, which expresses pseudotyped virions which contain LCMV glycoprotein inserted in the coat of said pseudotyped virions.

13. A process for the preparation of the retroviral packaging cell according to claim 5, comprising the step of contacting LCMV, under suitable conditions, with a retroviral packaging cell that comprises the genes **gag** and **pol** of a **retroviral vector** and a recombinant **retroviral vector** containing one or more transgenes selected from the group consisting of **marker** genes and genes encoding therapeutic proteins, whereby the infectious recombinant retroviral virions are pseudotyped with the LCMV glycoproteins expressed by said LCMV.

14. A process for the preparation of the retroviral packaging cell according to claim 5, comprising the step of introducing a plasmid vector expressing glycoproteins GP-1 and GP-2 of LCMV into a retroviral packaging cell that comprises the genes **gag** and **pol** of a **retroviral vector** and a recombinant **retroviral vector** containing one or more transgenes selected from the group consisting of **marker** genes and genes encoding therapeutic proteins, whereby the infectious recombinant retroviral particles are pseudotyped with the LCMV glycoproteins.

15. A process for the preparation of recombinant retroviral pseudotype virions, comprising the steps of performing the process according to claim 13 and cultivating the resulting retroviral packaging cells under conditions which are suitable for the production of recombinant retroviral pseudotype virions.

16. A process for the preparation of recombinant retroviral pseudotype virions, comprising the steps of performing the process according to

claim 11 and cultivating the resulting retroviral packaging cells under conditions which are suitable for the production of recombinant retroviral pseudotype virions.

17. A method for in vitro infection of cells and for the expression of a transgene in said cells, said method comprising contacting the cells with the retroviral packaging cells according to claim 5 or with cell culture supernatants of said retroviral packaging cells, wherein the contacting is performed under conditions which allow infection of the cells, and wherein the infected cells are cultured under conditions which allow expression of the transgene.

18. The retroviral packaging cell according to claim 5, wherein said therapeutic proteins are selected from the group consisting of a herpes simplex virus thymidine kinase (HSV-tk), a cytosine deaminase (CD) and a cytokine.

19. The retroviral packaging cell according to claim 5, wherein said therapeutic proteins are mdr-1 proteins.

L32 ANSWER 5 OF 16 USPATFULL on STN
2002:216834 Recombinant MVA virus, and the use thereof.
Sutter, Gerd, Munich, GERMANY, FEDERAL REPUBLIC OF
Ohlmann, Marion, Munich, GERMANY, FEDERAL REPUBLIC OF
Erfle, Volker, Munich, GERMANY, FEDERAL REPUBLIC OF
GSF-Forschungszentrum fur Umwelt und Gesenudheit GmbH, Oberschleisseim, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
US 6440422 B1 20020827
APPLICATION: US 1998-2443 19980102 (9) <--
PRIORITY: DK 1995-782 19950704
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to recombinant vaccinia viruses derived from the modified vaccinia virus Ankara (MVA) and containing and capable of expressing foreign genes which are inserted at the site of a naturally occurring deletion in the MVA genome, and the use of such recombinant MVA viruses for the production of polypeptides, e.g. antigens or therapeutic agents, or viral vectors for gene therapy, and the use of such recombinant MVA viruses encoding antigens as vaccines.
CLM What is claimed is:
1. A recombinant Modified Vaccinia Ankara (MVA) virus containing and capable of expressing at least one foreign gene inserted at a site of a naturally occurring deletion within the MVA genome, wherein the site of the naturally occurring deletion is not site III.

2. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 1 containing and capable of expressing at least one foreign gene inserted at the site of deletion II within the MVA genome.

3. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 1 wherein the foreign gene codes for a **marker**, a therapeutic agent or an antigenic determinant.

4. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 3 wherein the foreign gene codes for an antigenic determinant from a pathogenic virus, a bacteria, other microorganism, a parasite, and a tumor cell.

5. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 4 wherein the foreign gene codes for an antigenic determinant from Plasmodium Falciparum, Mycobacteria, Herpes virus, influenza virus, hepatitis, or human immunodeficiency viruses.

6. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 4 wherein the antigenic determinant is Human Immunodeficiency Virus nef

of human tyrosinase.

7. A recombinant MVA virus according to claim 6 which is Modified Vaccinia Ankara (MVA)-LAInef or MVA-human tyrosinase (hTYR).

8. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 1 wherein the foreign gene codes for T7 RNA polymerase.

9. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 8 which is MVA-T7 **pol**.

10. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 1 wherein the foreign gene is under transcriptional control of the vaccinia virus early/late promoter P7.5.

11. Recombinant Modified Vaccinia Ankara (MVA) viruses according to claim 1 wherein the viruses cannot replicate in human cells.

12. A recombinant Modified Vaccinia Ankara (MVA) virus containing and capable of expressing an Human Immunodeficiency Virus (**HIV**) nef gene inserted into the MVA genome.

13. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 12 which is MVA-LAInef.

14. A recombinant Modified Vaccinia Ankara (MVA) virus containing and capable of expressing a human tyrosinase gene inserted into the MVA genome.

15. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 14 which is MVA-human tyrosinase (hTYR.

16. A recombinant Modified Vaccinia Ankara (MVA) virus containing and capable of expressing at least one foreign gene inserted at a site of a naturally occurring deletion within the MVA genome, wherein the site of the naturally occurring deletion is selected from the group consisting of: site I, site II, site IV, site V and site VI.

17. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 16 wherein the foreign gene codes for a **marker**, a therapeutic agent or an antigenic determinant.

18. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 17 wherein the foreign gene codes for an antigenic determinant from a pathogenic virus, a bacteria, other microorganism, a parasite, and a tumor cell.

19. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 18 wherein the foreign gene codes for an antigenic determinant from Plasmodium Falciparum, Mycobacteria, Herpes virus, influenza virus, hepatitis, or human immunodeficiency viruses.

20. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 18 wherein the antigenic determinant is Human Immunodeficiency Virus nef or human tyrosinase.

21. The recombinant MVA virus according to claim 20 which is Modified Vaccinia Ankara (MVA)-LAInef or MVA-human tyrosinase (hTYR).

22. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 16 wherein the foreign gene codes for T7 RNA polymerase.

23. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 22 which is MVA-T7 **pol**.

24. The recombinant Modified Vaccinia Ankara (MVA) virus according to

claim 25 wherein the foreign gene is under transcriptional control of the vaccinia virus early/late promoter P7.5.

25. A recombinant Modified Vaccinia Ankara (MVA) virus containing and capable of expressing at least one foreign gene inserted at deletion site II of the MVA virus.

26. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 25 wherein the foreign gene codes for a **marker**, a therapeutic agent or an antigenic determinant.

27. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 26 wherein the foreign gene codes for an antigenic determinant from a pathogenic virus, a bacteria, other microorganism, a parasite, and a tumor cell.

28. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 27 wherein the foreign gene codes for an antigenic determinant from Plasmodium Falciparum, Mycobacteria, Herpes virus, influenza virus, hepatitis, or human immunodeficiency viruses.

29. The recombinant Modified Vaccinia Ankara (MVA) virus according to claims 27 wherein the antigenic determinant is Human Immunodeficiency Virus nef or human tyrosinase.

30. The recombinant MVA virus according to claim 29 which is Modified Vaccinia Ankara (MVA)-LAInef or MVA-human tyrosinase (hTYR).

31. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 25 wherein the foreign gene codes for T7 RNA polymerase.

32. A recombinant Modified Vaccinia Ankara (MVA) virus according to claim 31 which is MVA-T7 **pol**.

33. The recombinant Modified Vaccinia Ankara (MVA) virus according to claim 25 wherein the foreign gene is under transcriptional control of the vaccinia virus early/late promoter P7.5.

L32 ANSWER 6 OF 16 USPATFULL on STN
2002:133199 METHOD FOR THE TREATMENT OR DIAGNOSIS OF HUMAN PATHOLOGIES WITH DISSEMINATED OR DIFFICULT TO ACCESS CELLS OR TISSUES.
DREYFUS, PATRICK A., CLAMART, FRANCE
PARRISH, ELAINE, SAINT-DENIS, FRANCE
GARCIA, LUIS, SAINT-DENIS, FRANCE
CHOKRI, MOHAMED, STRASBOURG, FRANCE
BARTHOLEYNS, JACQUES, BURES-SUR-YVETTE, FRANCE
PELTEKIAN, ELISE, PARIS, FRANCE
US 2002068048 A1 20020606
APPLICATION: US 1997-924830 A1 19970905 (8) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Method for the treatment or diagnosis of pathologies either expressed in injured or pathological multiple sites in tissues or in the body or expressed in injured or pathological sites of tissues or cells in sites of the body, which are difficult to access, with said sites or areas in immediate proximity to said sites being the source of the release of chemotactic factors for endogenous macrophages, either spontaneously or upon suitable stimulation, wherein said treatment is carried out by administration to the body of an appropriate amount of exogenous monocyte derived cells, said monocyte derived cells being, in the case of treatment, loaded with corrective agents with respect to the pathologies to be treated, and with said monocyte derived cells having the properties of mobilization towards the source of the above-said released chemotactic factors and to target the cells present in the vicinity of the said released chemotactic factors, and in the case of

diagnosis, loaded with a marker enabling the detection of injured or pathological sites.

CLM What is claimed is:

1. Method for the treatment or diagnosis of pathologies either expressed in injured or pathological multiple sites in tissues or in the body or expressed in injured or pathological sites of tissues or cells in sites of the body, which are difficult to access, with said sites or areas in immediate proximity to said sites being the source of the release of chemotactic factors for endogenous macrophages, either spontaneously or upon suitable stimulation, wherein said treatment is carried out by administration to the body of an appropriate amount of exogenous monocyte derived cells, said monocyte derived cells being, in the case of treatment, loaded with corrective agents with respect to the pathologies to be treated, and with said monocyte derived cells having the properties of mobilisation towards the source of the above-said released chemotactic factors and to target the cells present in the vicinity of the said released chemotactic factors, and in the case of diagnosis, loaded with a **marker** enabling the detection of injured or pathological sites.

2. Method according to claim 1, wherein the treatment with said corrective agents consists in providing deficient elements, such as those responsible for or resulting from the pathology, or providing elements liable to inhibit or to kill abnormally stimulated cells, responsible for or resulting from the pathology.

3. Method according to claim 1 or 2, wherein the corrective agent is a chemical or a biological product such as a polypeptide, a growth factor, a nucleic acid, a gene or the product of a gene.

4. Method according to any of claims 1 to 3, wherein the monocyte derived cells are prepared ex vivo by culturing blood monocytes to obtain monocyte derived cargo cells and in particular mature phagocytes and enhancing their capability (signal linked to the membrane, carrier of product or information, phagocytosis and secretion) or/and loading said phagocytes with appropriate chemical or biological substances or transfecting them with a virus containing an appropriate gene of or with nucleic acids consisting in or containing an appropriate gene.

5. Method according to any of claims 1 to 4, wherein the chemotactic factors are released either by injured or pathological sites spontaneously resulting from the pathology or subsequent to a chemical or physical stimulation of the sites to be treated.

6. Method according to any of claims 1 to 5, wherein the multiple expressed sites result from disseminated cancers or from inflammatory diseases.

7. Method according to any of claims 1 to 5, wherein the injured or pathological sites difficult to access are: the central nervous system, the peripheral nervous and muscular systems and bones.

8. Method according to anyone of claims 1 to 5, wherein the pathologies treated by the method of the present invention include but are not limited to: For the central nervous system Genetic diseases such as: Adrenoleukodystrophy Spinal muscular atrophy Gaucher disease Huntington disease Sporadic diseases such as Alzheimer disease Parkinson disease Amyotrophic lateral sclerosis Multiple sclerosis Strokes Glioblastoma Cerebral metastasis Infection of the central nervous system Peripheral nervous and muscular system Genetic diseases such as: Duchenne disease, Becker disease Muscular dystrophies Non genetic diseases such as: Neuropathies and muscular necrosis from different origins (incl. trauma) Rheumatoid arthritis Atheromatosis Bone trauma or bone infection or degenerescence Pulmonary fibrosis.

9. Monocyte derived cells obtained by culturing blood mononuclear cells

to obtain monocytes derived large cells, containing a therapeutic agent for a given pathology corresponding to loaded chemical or biological substances such as peptides, polypeptides, proteins and nucleic acids or to virus or nucleic acids which have been transfected into said cells or to these cells loaded externally on the membrane with emitting signals, the said cells having one of more of the following properties: their preparation specifically induce an increased membrane expression level of chemotactic receptors, they are sensitive, particularly in vivo, to chemotactic factors released by sites of call or suffering cells, they have membrane a plasticity such that they can enter difficult injured sites to access such as the central nervous systems, they can rapidly reach sites of call, as soon as two hours to three days, particularly two to three days after systemic injection, they can accumulate into injured sites of call, they remain alive in the vicinity of the injured or pathological sites for several months, particularly at least up to about 4 months, their morphology becomes similar to the morphology of the cells normally present in the injured sites or pathological and they integrate the tissue cells of the injured or pathological sites, they can release the contained corrective agent in the sites of call, either constitutively or on demand by induction of secretion of said corrective agent.

10. Monocyte derived cells according to claim 9, loaded with chemical or biological substances introduced either by phagocytosis, pinocytosis or physical means such as electropulsation.

11. Monocyte derived cells according to claim 9, transduced using different defective viral vectors such as adenovirus, herpes simplex virus and **lentivirus**, **lentivirus**, thereby allowing the transduction of said monocyte derived cells to efficiently introduce therein a cassette containing nucleic sequences coding for a secretable therapeutic peptide, polypeptide or protein under the control of a specific promoter such as Pz.

12. Monocyte derived cells according to claim 9, transfected by introduction of a viral construction consisting of both a murine leukemia provirus (MuLV) containing a gene encoding a peptide, a polypeptide or protein of therapeutic interest and sequences encoding the helper genome allowing its mobilisation and the release of the viral construction at the injured sites.

13. Monocyte derived cells according to claim 12, either transduced sequentially with a) a defective viral vector (matrix vector), able to transduce post-mitotic cells, carrying the sequences encoding entirely the provirus defined in claim 12 (which carries the therapeutic gene), b) a defective viral vector (assembling vector), able to transduce post-mitotic cells, carrying a defective MuLvs **gag-pol**-env genome for transcomplementation allowing replication of the above-said provirus, or transduced by a single defective viral vector (master vector), able to transduce post-mitotic cells, carrying both the sequences encoding entirely the provirus defined in claim 12 (which carries the therapeutic gene under the control of an internal promoter Py) and a defective MuLvs **gag-pol**-env genome under the control of an internal promoter Pz, for ciscomplementation allowing replication and production of the above-said provirus.

14. Kit for the preparation of monocyte derived cells according to anyone of claims 9 to 13 comprising: culture means (bags and means) for the maturation of mononuclear cells into phagocytes, particularly macrophages, therapeutic agents to be introduced into the above-said phagocytes and means of introducing them to obtain monocyte derived cells.

15. Kit according to claim 14 containing one or more of the following compnents: means for viral transduction of said phagocytes with defective viral vectors to obtain monocyte derived cells, description

of physical (laser, puncture, irradiation . . .) and chemical means to induce the local signal when required, including the time schedule, reagents for the quality control of the viral transduction and of the monocyte derived cells, software for the standard operating procedures and traceability particularly of the following steps: culture of phagocytes, introduction of corrective agents, viral transduction and the recovery of the above-mentioned monocyte derived cells.

16. Pharmaceutical compositions containing as active substance monocytes derived cells according to anyone of claims 9 to 13 in association with a pharmaceutically acceptable vehicle.

L32 ANSWER 7 OF 16 USPATFULL on STN
2002:98897 RECOMBINANT VIRUS EXPRESSING FOREIGN DNA ENCODING FELINE CD80, FELINE CD86, FELINE CD28, FELINE CTLA-4 OR FELINE INTEFERON -GAMMA AND USES THEREOF.
WINSLOW, BARBARA J., DEL MAR, CA, UNITED STATES
COCHRAN, MARK D., CARLSBAD, CA, UNITED STATES
US 2002051792 A1 20020502
APPLICATION: US 1999-303040 A1 19990430 (9) <--
PRIORITY: US 1998-83870P 19980501 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention involves a recombinant virus which comprises at least one foreign nucleic acid inserted within a non-essential region of the viral genome of a virus, wherein each such foreign nucleic acid encodes a protein. The protein which is encoded is selected from the groups consisting of a feline CD28 protein or an immunogenic portion thereof, a feline cD80 protein or an immunogenic portion thereof, a feline CD86 protein or an immunogenic portion thereof, or a feline CTLA-4 protein or an immunogenic portion thereof. The protein is capable of being expressed when the recombinant virus is introduced into an appropiate host. The present invention also involves a recombinant virus further comprising a foreign nucleic acid encoding an immunogen derived from a pathogen. The present invention also comprises recombinant viruses which are capable of enhancing an immune response in a feline. The present invention also comprises recombinant viruses which are capable of suppressing an immune respons in a feline.

CLM What is claimed is:
1. A recombinant virus which comprises at least one foreign nucleic acid inserted within a non-essential region of the viral genome of a virus, wherein each such foreign nucleic acid (a) encodes a protein selected from the groups consisting of a feline CD28 protein or an immunogenic portion thereof; a feline CD80 protein or an immunogenic portion thereof; a feline Cd86 protein or an immunogenic portion thereof; or a feline CTLA-4 protein or an immunogenic portion thereof and (b) is capable of being expressed when the recombinant virus is introduced into an appropiate host.

2. The recombinant virus of claim 1 which comprises at least two foreign nucleic acids, each inserted whitin a non-essential region of the viral genome.

3. The recombinant virus of claim 2 which comprises at least three foreign nucleic acids, each inserted whitin a non-essential region of the viral genome.

4. The recombinant virus of claim 3 which comprises four foreign nucleic acids, each inserted whithin a non-essential region of the viral genome.

5. The recombinant virus of claim 1, wherein the virus is raccoonpox virus, a swinepox virus, or a feline herpesvirus.

6. The recombinant virus of any of claim 1-5 comprising more than one foreign nucleic acid, wherein each foreign nucleic acids is inserted

into the same nonessential region.

7. The recombinant virus of any claims 1-5 comprising more than one foreign nucleic acid wherein all such foreing nucleic acids are not inserted into the same nonessential region.

8. The recombinant virus of any of claim 1-7 further comprising a foreign nucleic acid encoding an immunogen derived from a pathogen.

9. The recombinant virus of claim 8, wherein the pathogen is a feline pathogen, a rabies virus, Chlamydia, Taxoplasmosis gondii, Dirofilaria immitis, a flea, or a bacterial pathogen.

10. The recombinant virus of claim 9, wherein the feline pathogen is feline immunodeficiency virus (**FIV**), feline leukemia virus (FeLV), feline infectious peritonitis virus (FIP), feline panleukopenia virus, feline calicivirus, feline reovirus type 3, feline rotavirus, feline coronavirus, feline syncytial virus, feline sarcoma virus, feline herpesvirus, feline Borna disease virus, or a feline parasite.

11. The recombinant virus of any of claim 1-7, wherein at least one foreign nucleic acid comprises a promoter for expressing the foreign nucleic acid.

12. The recombinant virus of any claims 1-7, wherein the expression of a least one foreign nucleic acids is under the control of a promoter endogenes to the virus.

13. The recombinant virus of any of claims 1-10 further comprising a foreign nucleic acid encoding a detectable marlar.

14. The recombinant virus of claim 13, wherein the detectable **marker** is E. coli beta galactosidase.

15. The recombinant virus of claim 10, wherein the immunogen from a feline pathogen is **FIV gag** protease, a **FIV** envelope protein, a FeLV **gag** protease, or a FeLV envelope protein.

16. The recombinant virus of any claims 1-7, wherein the virus is a feline herpesvirus and the a nonessential region is the glycoprotein G gene of feline herpes virus.

17. The recombinant feline herpesvirus of claim 12 designated S-FHV-031 (ATCC Accession No. VR-2604).

18. The recombinant virus of any of claim 1-7, wherein the virus is swinepox virus and the nonessential region is the larger Hind III to Bgl II subfragment of the Hind III M fragment of swinepox virus.

19. The recombinant feline swinepox of claim 14 designated S-SPV-246 (ATCC Accession No.VR-2603).

20. The recombinant virus of any of claims 1-7, wherein the portion of the CD28, CD80, or CD86 protein is the soluble portion of the protein.

21. The recombinant virus of any of claims 1-7, where the foreign nucleic acid encodes the feline CTLA-4 protein.

22. A vaccine which comprising an effective immunizing amount of the recombinant virus of any of claims 1-19 and a suitable carrier.

23. The vaccine of claim 22, wherein the effective immunizing amount of the recombinant virus is an amount between about 1×10^5 pfu/ml and about 1×10 cfu/ml.

24. The vaccine of claims 22 which further comprises an admixture with

the recombinant virus an effective immunizing amount of an a second immunogen.

25. A method for enhancing an immune response in a feline which comprises administering to the feline an effective immunizing amount of the recombinant virus of any of claims 1-19.

26. A method for immunizing a feline which comprising administering to the feline an effective immunizing amount of the recombinant virus of any of claims 1-19.

27. A method for suppressing an immune response in a feline which comprises administering to the feline any effective suppressing amount of the recombinant virus of claim 20 or 21.

28. The method of any of claims 24-26, wherein the administering comprises intravenous, subcutaneous, intramuscular, transmuscular, topical, oral, or intraperitoneal administration.

29. The method of claim 27, wherein the feline is the recipient of a transplanted organ or tissue or is suffering from an immune response.

30. A method for suppressing an immune response in a feline which comprises administering to the feline an antisense nucelic acid capable of hybridizing to and inhibiting translation of: (a) a feline CD28 mRNA transcript, (b) a feline CD80 transcript, or (c) a feline CD86 mRNA transcript the antisense nucleic acid being present in an amount effective to inhibit translation and thus suppress the immune response in the feline.

31. A method for reducing or abrogating a tumor in a feline which comprises administering to the tumor in the feline a recombinant virus of claim 1, wherein the nucleic acid encodes a feline CD80 protein, a feline CD86 protein or a combination thereof in an amount effective reduce or abrogate the tumor.

32. The method of claim 31, wherein the recombinant virus further comprises, and is capable of expressing, a feline tumor associated antigen and the administration is effected systemically.

33. The recombinant virus of claim 1, further comprising a nucleic acid encoding feline immunodeficiency virus genome or a portion thereof.

34. The recombinant virus of claim 1, further comprising a nucleic acid encoding feline leukemia virus genome or a portion thereof.

35. The recombinant virus of claim 33 or 34, further comprising a nucleic acid encoding feline IL12, p35 or p40.

36. A vaccine which comprises an effective immunizing amount of the recombinant virus of claim 33 or 34 and a suitable carrier.

L32 ANSWER 8 OF 16 USPATFULL on STN
2002:92287 FELINE IMMUNODEFICIENCY VIRUS GENE THERAPY VECTORS.
JOHNSTON, JULIE C., WILMINGTON, DE, UNITED STATES
SAUTER, SYBILLE L., DEL MAR, CA, UNITED STATES
HSU, DAVID CHI-TANG, SAN DIEGO, CA, UNITED STATES
SHERIDAN, PHILIP LEE, SAN DIEGO, CA, UNITED STATES
HARDY, STEPHEN F., SAN FRANCISCO, CA, UNITED STATES
DUBENSKY, THOMAS W., JR., PIEDMONT, CA, UNITED STATES
YEE, JIING-KUAN, DEL MAR, CA, UNITED STATES
US 2002048805 A1 20020425
APPLICATION: US 1999-231235 A1 19990115 (9) <--
PRIORITY: US 1998-71731P 19980116 (60)
US 1998-86825P 19980526 (60)

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are gene therapy vectors based upon the feline immunodeficiency virus, as well as related packaging cell lines, methods for production, and methods of use.

CLM What is claimed is:

1. An **FIV** vector, comprising a 5' **FIV** LTR, a primer binding site, one or more heterologous sequence(s) operably linked to a promoter element, an origin of second stand DNA synthesis and a 3' **FIV** LTR.

2. The **FIV** vector according to claim 1 wherein said primer binding site is a tRNA binding site.

3. The **FIV** vector according to claim 1, further comprising a packaging signal.

4. The **FIV** vector of claim 1 wherein the **FIV** vector contains less than 20 consecutive nucleotides occurring within a **gag/pol** or env sequence of a retrovirus.

5. The **FIV** vector of claim 2 wherein the **FIV** vector contains less than 15 consecutive nucleotides occurring within a **gag/pol** or env sequence of a retrovirus.

6. The **FIV** vector of claim 2 wherein the **FIV** vector contains less than 10 consecutive nucleotides occurring within a **gag/pol** or env sequence of a retrovirus.

7. The **FIV** vector of claim 2 wherein the **FIV** vector contains less than 6 consecutive nucleotides occurring within a **gag/pol** or env sequence of a retrovirus

8. The **FIV** vector according to claim 1 wherein the promoter element is a tissue-specific promoter.

9. The **FIV** vector according to claim 1 wherein the promoter element is a viral promoter.

10. The **FIV** vector according to claim 9 wherein the viral promoter is a CMV, SV40, PGK or **HIV**-1 promoter.

11. The vector according to claim 1 wherein at least one of the 5' **FIV** LTR, the 3' **FIV** LTR or the **FIV** LTR promoter is comprised of at least 45% of a wild type sequence.

12. The vector according to claim 1, further comprising at least one non-**FIV** promoter or promoter/enhancer.

13. The vector according to claim 12 wherein said promoter or promoter/enhancer is inducible.

14. The **FIV** vector according to claim 1 wherein at least one gene of interest is a **marker** gene.

15. The **FIV** vector according to claim 1 wherein at least one gene of interest is selected from a gene encoding cytokines, factor VIII, factor IX, LDL receptor, prodrug activating enzymes, trans-dominant negative viral or cancer-associated proteins, angiogenesis and anti-angiogenesis factors, CFTR, β-glucuronidase, sarcoglycans, glucokinase, NGF, VEGF, FGF, PDGF, IGF, GC, BDNF, and tyrosine hydroxylase.

16. The **FIV** vector according to claim 1 further comprising an internal ribosome entry site.

17. The **FIV** vector according to claim 1 wherein the promoter is operably linked to two genes of interest which are separated by less

18. The **FIV** vector according to any one of claims 1-17 further including at least one RNA export element.

19. The **FIV** vector according to claim 18 wherein the RNA export element is selected from MPMV, HBV, RSV and **lentiviral** Rev-responsive-elements.

20. The **FIV** vector according to any one of claims 1-17 wherein the **FIV** vector lacks an RNA export element.

21. A packaging expression cassette comprising a promoter operably linked to a sequence encoding an **FIV gag** region.

22. A packaging expression cassette comprising a promoter operably linked to a sequence encoding an **FIV pol** region.

23. A packaging expression cassette comprising a promoter operably linked to a sequence encoding an **FIV gag/pol** region.

24. The packaging expression cassette of claim 22 wherein the **gag/pol** region includes a dUTPase gene.

25. The packaging expression cassette of claim 22 wherein the **FIV gag/pol** region is a partial sequence of a wild type **FIV gag/pol** region.

26. A packaging expression cassette according to claim any one of claims 21, 22, or 23, further comprising an element selected from the group consisting of vif, ORF 2 or rev.

27. A packaging expression cassette according to any one of claims 22-26 further containing at least one RNA export element.

28. A rev expression cassette, comprising a promoter operably linked to a sequence comprising at least one of vif, rev or ORF 2.

29. An **FIV** envelope expression cassette comprising a promoter operably linked to an **FIV** envelope gene.

30. A host cell containing an expression cassette according to anyone of claims 22-29.

31. A packaging cell, comprising one or more expression cassettes that direct the expression of **FIV gag**, **FIV pol**, and a viral envelope.

32. The packaging cell line according to claim 31 wherein one expression cassette directs the expression of **FIV gag**, a second expression cassette directs the expression of **FIV pol**, and a third expression cassette directs the expression of a first viral envelope.

33. The packaging cell line according to claim 32, further comprising a fourth expression cassette that directs the expression of a second viral envelope.

34. The packaging cell line according to claim 31 wherein said packaging cell line further comprises a sequence encoding one or more of vif rev or ORF 2.

35. The packaging cell line according to claim 31 wherein at least one expression cassette is stably integrated.

36. The packaging cell line according to claim 31 wherein said cell line, upon introduction of a **FIV** vector construct, produces particles at a concentration of greater than 10^3 cfu/ml.

37. The packaging cell line according to any one of claims 31 to 36, wherein at least one promoter is inducible.

38. The packaging cell line according to claim 31 wherein said envelope is VSV-G.

39. The packaging cell line according to claim 31 wherein said cell line, upon introduction of a **FIV** vector, produces particles that are free of replication competent virus.

40. The packaging cell line according to claim 31 wherein said cell line is of feline or human origin.

41. The packaging cell line according to any one of claims 31 to 39 wherein said packaging cell line contains at least one expression cassette with at least one RNA export element.

42. **FIV** vector particles that are substantially free from wild-type **FIV** vector particles.

43. The **FIV** vector particle according to claim 42 wherein said particle has a heterologous viral envelope.

44. The **FIV** vector particle according to claim 42 wherein said particle has an external heterologous protein.

45. A method for producing **FIV** vector particles, comprising introducing into a host cell an **FIV** vector construct, and one or more expression cassettes that direct the expression of **FIV gag**, **FIV pol** and a viral envelope.

46. The method according to claim 45, further comprising one or more expression cassettes that direct the expression of vif, rev, or, ORF2.

47. A method of administering a selected nucleic acid molecule to a host, comprising administering to said host an **FIV** vector particle which directs the expression of said selected nucleic acid molecule.

48. The method according to claim 47, wherein said **FIV** vector particle is administered to the brain, spinal cord, bone marrow, eyes, nasal epithelium, lung, vasculature, skin, heart, liver, spleen, tumor.

49. The method according to claim 47, wherein said **FIV** vector particle is administered to cells ex vivo.

50. A method for concentrating **FIV** vector particles, comprising precipitating vector particles produced according to the method of claim 45.

51. A method for concentrating **FIV** vector particles, comprising centrifuging vector particles produced according to the method of claim 45.

L32 ANSWER 9 OF 16 USPATFULL on STN
2001:178884 Construction of retroviral producer cells from adenoviral and **retroviral vectors.**
Lin, Xinli, Edmond, OK, United States
Tang, Jordan J. N., Edmond, OK, United States
Oklahoma Medical Research Foundation, Oklahoma City, OK, United States (U.S. corporation)
US 6303380 B1 20011016
APPLICATION: US 1999-301846 19990429 (9) <--
PRIORITY: US 1998-83511P 19980429 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A combination of adenoviral and **retroviral vectors** used to construct second generation packaging cells that deliver marker genes to target cells is described. A vector based upon Moloney murine leukemia virus (MLV) was used to deliver marker genes, and an adenovirus-based delivery system was used to deliver MLV structural genes (gagpol and env) to cultured cells. The procedure transformed the cells into new retroviral producer cells, which generate replication-incompetent retroviral particles in the culture supernatant for transferring marker genes to target cells. The titer of the retroviral-containing supernatant generated from the second generation producer cells reached above 105 cfu/ml which is comparable to the MLV-based producer cell lines currently used in human gene therapy trials. The vector and procedures are adaptable for experimental human gene therapy in which the new producer cells are transplanted into patients for continuous gene transfer.

CLM What is claimed is:

1. A method of making a producer cell that delivers a **marker** gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell, comprising the steps: using a **retroviral vector** to deliver a **marker** or therapeutic gene to the human primary cell; and using a single adenoviral-based vector to deliver the **gag**, **pol** and env structural genes to the human primary cell, thereby making a producer cell.

2. The method of claim 1, wherein the **retroviral vector** is a Moloney murine leukemia virus (MLV) based vector to deliver a **marker** or therapeutic gene to the human primary cell.

3. The method of claim 1, wherein the **retroviral vector** is a **lentivirus**.

4. The method of claim 1 wherein the **gag**, **pol** and env structural genes are from Moloney murine leukemia virus.

5. The method of claim 3 wherein the **lentivirus** is human immunodeficiency virus.

6. The method of claim 3, wherein the structural **gag**, **pol** and env structural genes are from human immunodeficiency virus-1.

7. A producer cell that delivers a **marker** gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell transfected with a first **retroviral vector** delivering a **marker** or therapeutic gene to the human primary cell; and a second single adenoviral-based vector delivering the **gag**, **pol** and env structural genes to the human primary cell.

8. A system for making a producer cell that delivers a **marker** gene or therapeutic gene to a target cell, wherein the producer cell is a human primary cell, the system comprising: a first **retroviral vector** for delivering a **marker** or therapeutic gene to the human primary cell; and a second adenoviral-based vector for delivering the **gag**, **pol** and env structural genes to the human primary cell.

9. The system of claim 8 wherein the first **retroviral vector** is a Moloney murine leukemia virus (MLV) based vector to deliver a **marker** or therapeutic gene to the human primary cell.

10. The system of claim 8, wherein the first **retroviral vector** is a **lentivirus**.

11. The system of claim 8 wherein the **gag**, **pol** and env structural genes are from Moloney murine leukemia virus.

12. The system of claim 10 wherein the **lentivirus** is human immodeficiency virus.

L32 ANSWER 10 OF 16 USPATFULL on STN
2001:110143 ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS FOR PREPARING ARTIFICIAL CHROMOSOMES.
HADLACZKY, GYULA, SZAMOS, Hungary
SZALAY, ALADAR A., HIGHLAND, CA, United States
US 2001008025 A1 20010712
APPLICATION: US 1998-96648 A1 19980612 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for preparing cell lines that contain artificial chromosomes, methods for preparation of artificial chromosomes, methods for purification of artificial chromosomes, methods for targeted insertion of heterologous DNA into artificial chromosomes, and methods for delivery of the chromosomes to selected cells and tissues are provided. Also provided are cell lines for use in the methods, and cell lines and chromosomes produced by the methods. In particular, satellite artificial chromosomes that, except for inserted heterologous DNA, are substantially composed of heterochromatin are provided. Methods for use of the artificial chromosomes, including for gene therapy, production of gene products and production of transgenic plants and animals are also provided.

CLM What is claimed is:

1. A method for producing an artificial chromosome, comprising: introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable **marker**; growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA; and selecting a cell that comprises a satellite artificial chromosome.

2. The method of claim 1, further comprising, isolating the SATAC.

3. A SATAC produced by the method of claim 1.

4. The method of claim 1, wherein the DNA fragment comprises a sequence of nucleotides that targets the fragment to the heterochromatic region of a chromosome.

5. The method of claim 4, wherein the targeting sequence of nucleotides is satellite DNA.

6. A cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 1.

7. The cell of claim 6, wherein the artificial chromosome is a satellite artificial chromosome.

8. An isolated substantially pure satellite artificial chromosome.

9. The SATAC of claim 8 that is a megachromosome, comprising about 50 to about 450 megabases.

10. The SATAC of claim 9, comprising about 250 to about 400 Mb.

11. The SATAC of claim 9, comprising about 150 to about 200 Mb.

12. The SATAC of claim 9, comprising about 90 to about 120 Mb.

13. The SATAC of claim 9, comprising about 60 to about 100 Mb.

14. The method of claim 1, further comprising introducing a fragmentation vector, whereby megachromosomes in the cells of step B are reduced in size to resulting in cells that contain SATACs that are about 15 to about 50 Mb.

15. The method of claim 14, further comprising selecting a cell that

comprises a satellite artificial chromosome that comprises about 15 to about 50 Mb.

16. A cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 14.

17. A cell containing an artificial chromosome, wherein the artificial chromosome is produced by the method of claim 15.

18. The cell of claim 15, wherein the artificial chromosome is a SATAC comprising about 15 to about 50 Mb.

19. An isolated substantially pure satellite artificial chromosome that comprises about 15 to about 50 Mb.

20. The method of claim 1, further comprising isolating the SATAC from the cell.

21. The method of claim 1, wherein isolation is effected by: isolating metaphase chromosomes; staining the chromosomes with DNA sequence-specific dyes; and separating the SATACs from other chromosomes in the cells by flow cell sorter.

22. A method for producing an artificial chromosome, comprising: introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable **marker**, growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA, selecting from among those cells, a cell that has a chromosome that comprises a de novo centromere.

23. The method of claim 22, further comprising isolating that cell with the chromosome that comprises the de novo centromere, and growing the cell under conditions whereby a cell with a sausage chromosome is produced.

24. The method of claim 23, further comprising isolating the cell with the sausage chromosome; and growing the cell under conditions whereby a first SATAC is produced.

25. The method of claim 24, further comprising: introducing a fragmentation vector that is targeted to the first SATAC; growing the cells; and selecting a cell that comprises a second SATAC, wherein the second SATAC is smaller than the first SATAC.

26. The method of claim 22, wherein the selected cell has a dicentric chromosome comprising the de novo centromere.

27. The method of claim 22, wherein the selected cell has a formerly dicentric chromosome and a minichromosome comprising the de novo centromere.

28. The method of claim 22, wherein the selected cell has a formerly dicentric chromosome.

29. A method for producing an artificial chromosome, comprising introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable **marker**; growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA; selecting from among those cells a cell that has produced a dicentric chromosome; and growing that cell under selective conditions, whereby a cell that contains a chromosome comprising a heterochromatic arm is produced.

30. The method of claim 29, further comprising selecting the cell with the chromosome comprising the heterochromatic arm and growing it in the presence of an agent that destabilizes the chromosome.

31. The method of claim 30, further comprising identifying cells that contain a heterochromatic chromosome that is about 50 to about 400 Mb.

32. A method for producing a transgenic animal, comprising introducing a satellite artificial chromosome into an embryonic cell.

33. The method of claim 32, wherein the embryonic cell is a stem cell.

34. The method of claim 32, wherein the embryonic cell is in an embryo.

35. The method of claim 32, wherein the SATAC comprises heterologous DNA that encodes a therapeutic product.

36. The method of claim 32, wherein the product is the cystic fibrosis transmembrane regulatory protein, an anti-**HIV** ribozyme, or a tumor suppressor gene.

37. The method of claim 32, wherein the anti-**HIV** ribozyme is an anti-**gag** ribozyme, and the tumor suppressor gene is p53.

38. The method of claim 32, wherein the product comprises an antigen that upon expression induces a immunoprotective response against a pathogen in the transgenic animal.

39. The method of claim 32, wherein the product comprises a plurality of antigens that upon expression induce an immuno-protective response against a plurality of pathogens.

40. The method of claim 32, wherein the transgenic animal is a fish, insect, reptile, amphibians, arachnid or mammal.

41. The method of claim 32, wherein the SATAC is introduced by cell fusion, microinjection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer.

42. A transgenic animal produced by the method of claim 32.

43. A method of for producing a transgenic plant or animal, comprising: introducing a DNA fragment into a cell, wherein the DNA fragment comprises a selectable **marker;** growing the cell under selective conditions to produce cells that have incorporated the DNA fragment into their genomic DNA; and selecting a cell that comprises a minichromosome that is about 10 Mb to about 50 Mb that comprises the selectable **marker** and euchromatin; isolating the minichromosome and introducing it into a plant or animal cell.

44. The method of claim 43, wherein: after selecting the cell, DNA encoding a gene product or products is introduced into the cell, and the cell is grown under selective conditions, whereby cells comprising minichromosomes comprising the DNA encoding the gene product(s) are produced.

45. The method of claim 2, wherein: after selecting the cell,DNA encoding a gene product or products is introduced into the cell, and the cell is grown under selective conditions, whereby cells comprising SATACS that comprise the DNA encoding the gene product(s) are produced.

46. A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome into a plant cell; and culturing the cell under conditions whereby a plant is generated.

47. The method of claim 46, wherein the SATAC is introduced by protoplast fusion, microinjection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer.

48. A method for producing a gene product(s), comprising introducing a satellite artificial chromosome into a cell; and culturing the cell under conditions whereby the gene product(s) is (are) expressed.

49. The method of claim 48, wherein the gene product is produced by expression of a series of genes that encode a metabolic pathway; and the SATAC comprises each of these genes.

50. A method for cloning a centromere from an animal or plant, comprising: preparing a library of DNA fragments that comprise the genome of the plant or animal; introducing the each of the fragments into mammalian satellite artificial chromosomes, wherein: each SATAC comprises a centromere from a different species from the selected plant or animal, and a selectable **marker**; introducing each of the SATACs into the cells and growing the cells under selective conditions; identifying cells that have a SATAC; and selecting from among those cells any that have a SATAC comprising a centromere that differs from the centromeres in the original SATAC.

51. A cell line having the identifying characteristics of any of TF1004G19C5, 19C5xHa4, H1D3 and G3D5, which have been deposited at the ECACC under Accession Nos. 96040926, 96040927, 96040929, and 96040928, respectively.

52. A cell line, comprising a megachromosome that comprises about 50-400 Mb.

53. A cell line of claim 52, wherein the megachromosome comprises about 250 to about 400 Mb.

54. A cell line of claim 52, wherein the megachromosome comprises about 150 to about 200 Mb.

55. A cell line of claim 52, wherein the megachromosome comprises about 90 to about 120 Mb.

56. A cell line of claim 52, wherein the megachromosome comprises about 60 to about 100 Mb.

57. A method for gene therapy, comprising: introducing a SATAC that comprises DNA therapeutic product into a target cell; and introducing the resulting target cells into a host animal.

58. The method of claim 57, wherein the target cells are lymphocytes, stem cells or nerve cells.

59. The method of claim 43, wherein the minichromosome is the minichromosome present in the cell line EC3/7C5.

60. The method of claim 43, wherein the chromosome is the A neo-chromosome in the cell line KE1 2/4.

61. An artificial chromosome produced by the method of claim 1 that comprises more euchromatin than heterochromatin.

62. The artificial chromosome of claim 61 that is between about 20 Mb and about 200 Mb.

63. The artificial chromosome of claim 61 that is between about 100 Mb and about 200 Mb.

L32 ANSWER 11 OF 16 USPATFULL on STN
2001:55763 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
Finer, Mitchell H., San Carlos, CA, United States

Bell, Thomas D., San Francisco, CA, United States
Zsebo, Krisztina M., Woodside, CA, United States
Cooke, Keegan, Palo Alto, CA, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6218187 B1 20010417
APPLICATION: US 1999-266596 19990311 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.
CLM What is claimed is:

1. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

2. The method of claim 1, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

3. The method of claim 1, wherein said foreign gene encodes a chimeric T cell receptor.

4. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of; A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent

helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (**HIV**) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

5. The method of claim 4, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

6. The method of claim 4, wherein said foreign gene encodes a chimeric T cell receptor.

7. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding **gag** and **pol** proteins and a selectable **marker** wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

8. The method of claim 7, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

9. The method of claim 7, wherein said foreign gene encodes a chimeric T cell receptor.

10. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by transient transfection comprising the steps of: A) transient transfection of a first population of

mammalian cells stably transfected with at least one expression vector encoding **gag**, **pol** and env proteins and a selectable **marker** wherein the expression of **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding adhesion molecules or antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian hematopoietic stem cells on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

11. The method of claim 10, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

12. The method of claim 10, wherein said foreign gene encodes a chimeric T cell receptor.

13. A method to transduce mammalian hematopoietic stem cells with retroviral supernatants produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding adhesion molecules or antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian hematopoietic stem cells on said culture plates; and D) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian hematopoietic stem cells, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

14. The method of claim 13, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

15. The method of claim 13, wherein said foreign gene encodes a chimeric T cell receptor.

16. The method of any one of claims 1, 4, 7, 10 or 13 wherein said adhesion molecules are selected from the group consisting of fibronectin and CS-1.

17. The method of any one of claims 1, 4, 7, 10 or 13 wherein said antibodies to adhesion molecules are selected from the group consisting of antibodies to VLA-4, VLA-5, CD29, CD11a, CD11b and CD44.

18. A method to transduce mammalian T and B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site; and (ii) a **retroviral vector** encoding a

foreign gene to produce replication defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

19. The method of claim 18, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

20. The method of claim 18, wherein said foreign gene encodes a chimeric T cell receptor.

21. The method of claim 18, further comprising infecting a second population of mammalian target cells with the supernatant from said mammalian cells of claim 18 to transduce said target cells with a foreign gene.

22. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells that can produce virus with: (i) two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging the helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding the **gag** and **pol** proteins of ectropic MMLV or GALV and a second retroviral helper sequence comprises a cDNA encoding the envelope protein, and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

23. The method of claim 22, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

24. The method of claim 22, wherein said foreign gene encodes a chimeric T cell receptor.

25. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient cotransfection of a first population of mammalian cells stably transfected with an expression vector encoding the **gag** and **pol** proteins and a selectable **marker** wherein the expression of **gag** and

pol proteins is stable in the absence of a selective agent with (i) one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, and encoding an envelope protein; and (ii) a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

26. The method of claim 25, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

27. The method of claim 25, wherein said foreign gene encodes a chimeric T cell receptor.

28. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by transient transfection comprising the steps of: A) transient transfection of a first population of mammalian cells stably transfected with at least one expression vector encoding the **gag**, **pol** and env proteins and a selectable **marker** wherein the expression of the **gag**, **pol** and env proteins is stable in the absence of a selective agent with a **retroviral vector** encoding a foreign gene to produce replication-defective recombinant **retroviral vectors** carrying said foreign gene in said first population of mammalian cells; B) separation of said first population of mammalian cells from cell supernatant; C) adding antibodies to adhesion molecules to culture plates; D) growing a second population of mammalian T or B lymphocytes on said culture plates; and E) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

29. The method of claim 28, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

30. The method of claim 28, wherein said foreign gene encodes a chimeric T cell receptor.

31. A method to transduce mammalian T or B lymphocytes with **retroviral vectors** produced by stable mammalian viral producer cells comprising the steps of: A) separation of said first population of stable mammalian viral producer cells from cell supernatant; B) adding antibodies to adhesion molecules to culture plates; C) growing a second population of mammalian T or B lymphocytes on said culture plates; and D) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying said foreign gene with said second population of mammalian T or B lymphocytes, to transduce said second population of cells with said foreign gene, whereby target cells transduced with said foreign gene are obtained.

32. The method of claim 31, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

33. The method of claim 31, wherein said foreign gene encodes a chimeric T cell receptor.

34. The method of any one of claims 18, 22, 25, 28 or 31 wherein said antibodies to adhesion molecules is selected from the group consisting of antibodies to LFA-1, CD-2, CD40 and gp39.

35. The method of claims 1, 4, 7, 10, 13, 18, 22, 25, 28 or 31, wherein the first population of mammalian cells comprises a human cell.

36. An improved method to efficiently transduce mammalian cells with a retroviral supernatant, comprising the steps of: i) growing said population of mammalian cells on culture plates; and ii) incubating said supernatant containing replication-defective recombinant **retroviral vectors** carrying a foreign gene with said population of mammalian cells, to transduce said population of mammalian cells with said foreign gene, whereby target cells efficiently transduced with said foreign gene are obtained, wherein the improvement comprises adding antibodies to adhesion molecules present on said population of mammalian cells to culture plates.

37. The target cell of claim 36, wherein said foreign gene is selected from the group consisting of genes encoding growth factors, lymphokines, hormones and coagulation factors.

38. The target cell of claim 37, wherein said foreign gene encodes a chimeric T cell receptor.

39. The target cell of claim 38, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

40. The method of claim 3, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

41. The method of claim 6, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

42. The method of claim 9, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

43. The method of claim 12, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a

sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

44. The method of claim 15, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

45. The method of claim 20, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

46. The method of claim 24, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

47. The method of claim 27, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

48. The method of claim 30, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

49. The method of claim 33, wherein said chimeric T cell receptor is a receptor encoded by a DNA sequence comprising in reading frame: a sequence encoding a signal sequence; a sequence encoding a non-MHC restricted extracellular surface membrane protein domain binding specifically to at least one ligand; a sequence encoding a transmembrane domain; and a signal sequence encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system.

50. The method of claim 35, wherein said human cell is a 293 cell.

51. The method of claim 21, wherein said target cells are lymphocytes or hematopoietic stem cells.

L32 ANSWER 12 OF 16 USPATFULL on STN
2000:47084 Method for production of high titer virus and high efficiency retroviral mediated transduction of mammalian cells.
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Dull, Thomas L., San Francisco, CA, United States

Qin, Lu, Lutherville, MD, United States
Farson, Deborah A., Oakland, CA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6051427 20000418
APPLICATION: US 1995-517488 19950821 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides a novel retroviral packaging system, in which retroviral packaging plasmids and packagable vector transcripts are produced from high expression plasmids after stable or transient transfection in mammalian cells. High titers of recombinant retrovirus are produced in these transfected mammalian cells and can then transduce a mammalian target cell by cocultivation or supernatant infection. The methods of the invention include the use of the novel retroviral packaging plasmids and vectors to transduce primary human cells, including T cells and human hematopoietic stem cells, with foreign genes by cocultivation or supernatant infection at high efficiencies. The invention is useful for the rapid production of high titer viral supernatants, and to transduce with high efficiency cells that are refractory to transduction by conventional means.

CLM What is claimed is:

1. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-imcompetent retroviral genome encoding in trans all virion proteins required and for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said helper DNA sequence codes for ecotropic Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (**HIV**) **gag** and **pol**, and an envelope protein or chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, **HIV**, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

2. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (**HIV**), and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, and wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein obtained from virus selected from the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, **HIV**, vesicular

and HTLV type II.

3. The stable packaging cell line comprising helper sequences encoding **gag** and **pol** proteins designated 35.32.

4. A human embryonic kidney cell stably transfected with an expression vector encoding **gag** and **pol** proteins and a selectable **marker**, wherein the expression of **gag** and **pol** proteins is stable in the absence of a selective agent.

5. The human embryonic kidney cell of claim 4 wherein the **gag** and **pol** are derived from Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (**HIV**).

6. The human embryonic kidney cell of claim 4 wherein said cell is either 293 or tsa54.

7. A human embryonic kidney cell stably transfected with two expression vectors wherein the first expression vector encodes **gag** and **pol** proteins and the second expression vector encodes an envelope protein.

8. The human embryonic kidney cell of claim 7 wherein said cell is either 293 or tsa54 and said gal and **pol** proteins and derived from Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or human immunodeficiency virus (**HIV**).

9. The human embryonic kidney cell of claim 7 or 10 wherein said envelope protein is derived from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, **HIV**, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II.

10. The human embryonic kidney cell of claim 9, wherein said envelope protein is comprised of sequences from two or more of said viruses.

11. A stable packaging cell line comprising helper sequences encoding **gag**, **pol** and envelope proteins designated 37S2.8.

12. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising one retroviral helper DNA sequence derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequence lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking the both the psi function sequence responsible for packaging helper genome and the 3'LTR, and encoding a foreign enhancer and/or promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein said foreign enhancer is the RSV enhancer and promoter.

13. A retroviral packaging plasmid for the production of high titers of recombinant retrovirus in human cells comprising two retroviral helper DNA sequences derived from a replication-incompetent retroviral genome encoding in trans all virion proteins required for packaging a replication-incompetent **retroviral vector** and for producing virion proteins for packaging said replication-incompetent **retroviral vector** at high titer, without the production of replication-competent helper virus, said retroviral DNA sequences lacking the region encoding the native enhancer and/or promoter of the viral 5' LTR of said virus and lacking both the psi function sequence responsible for packaging helper genome and the 3' LTR, and encoding a foreign enhancer and/or

promoter functional in a selected mammalian cell, and a foreign polyadenylation site, wherein a first retroviral helper sequence comprises a cDNA sequence encoding **gag** and **pol** proteins of ecotropic Moloney murine leukemia virus (MMLV), gibbon ape leukemia virus (GALV) or immunodeficiency virus (**HIV**) and a second retroviral helper sequence comprises a cDNA encoding an envelope protein, wherein said second retroviral helper DNA sequence codes for an envelope protein or a chimeric envelope protein selected from virus of the group consisting of xenotropic murine leukemia virus, amphotropic murine leukemia virus, ecotropic murine leukemia virus, polytropic murine leukemia virus, 10A1 murine leukemia virus, GALV, **HIV**, vesicular stomatitis virus G protein, human T cell leukemia virus (HTLV) type I and HTLV type II; and wherein said foreign enhancer is the RSV enhancer and promoter.

L32 ANSWER 13 OF 16 USPATFULL on STN
1999:24450 Identification of compounds that modulate HIV-1 vpr protein activity
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US 5874225 19990223
APPLICATION: US 1993-19601 19930219 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is directed toward methods of identifying compounds which inhibit the human immunodeficiency virus (HIV) viral protein R (Vpr) from stimulating the differentiation of undifferentiated cells. This invention takes advantage of the observation that cell lines from rhabdomyosarcomas, which are tumors of muscle origin, have been used as models of CD4-independent HIV infection. These cell lines can be induced to differentiate in vitro. The vpr gene of HIV-1 is sufficient for the differentiation of the human rhabdomyosarcoma cell line TE671. Differentiated cells are characterized by great enlargement, altered morphology, lack of replication, and high level expression of the muscle-specific protein myosin. Morphological differentiation and inhibition of proliferation of other transformed cell lines following vpr expression was also observed. This invention also relates toward methods of identifying compounds which inhibit HIV Vpr binding to Gag. These screening methods should facilitate the identification and development of antiviral agents.
CLM What is claimed is:
1. An in vitro method of identifying compounds that are capable of inhibiting **HIV**-1 Vpr-mediated differentiation of undifferentiated cells comprising the following steps: (I) contacting undifferentiated cells with **HIV**-1 Vpr in the presence or absence of a test compound; and, (ii) determining whether said cells cease proliferating and display cellular differentiation **markers** in the presence or absence of a test compound; wherein the presence of cellular proliferation and absence of differentiation **markers** in the test sample is indicative of said compound being capable of inhibiting **HIV**-1 Vpr-mediated differentiation of undifferentiated cells.

2. The method of claim 1 wherein said undifferentiated cells are selected from the group consisting of: solid muscle tumor alveolar rhabdomyosarcoma cell line RD, solid muscle tumor alveolar rhabdomyosarcoma cell line TE671, osteosarcoma cell line D17, osteosarcoma cell line MG63, osteosarcoma cell line HOS-TE86, myeloid lineage cell line KG-1, myeloid lineage cell line THP-1, myeloid lineage cell line PLB973, human glioblastoma cell line U-138MG, human glioblastoma/astrocytoma cell line U373MG, and human glioblastoma/astrocytoma cell line U87-MG.

3. An in vitro method of identifying compounds that are capable of

inhibiting HIV-1 Vpr-mediated suppression of cellular proliferation comprising the following steps: (I) contacting proliferating cells with **HIV**-1 Vpr in the presence or absence of a test compound; and, (ii) determining whether said cells cease proliferating in the presence or absence of said test compound; wherein the presence of cellular proliferation in the test sample is indicative of said compound being capable of inhibiting **HIV**-1 Vpr-mediated suppression of cellular proliferation.

4. The method of claim 3 wherein said proliferating cells are selected from the group consisting of: solid muscle tumor alveolar rhabdomyosarcoma cell line RD, solid muscle tumor alveolar rhabdomyosarcoma cell line TE671, osteosarcoma cell line D17, osteosarcoma cell line MG63, osteosarcoma cell line HOS-TE86, myeloid lineage cell line KG-1, myeloid lineage cell line THP-1, myeloid lineage cell line PLB973, human glioblastoma cell line U-138MG, human glioblastoma/astrocytoma cell line U373MG, and human glioblastoma/astrocytoma cell line U87-MG.

5. An in vitro method for the identification of compounds capable of inhibiting **HIV**-1 Vpr binding to **HIV**-1 **Gag** comprising the following steps: (I) contacting, in the presence or absence of a test compound, **HIV**-1 Vpr and **Gag**; and (ii) determining the level of binding between **HIV**-1 Vpr and **Gag**, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting **HIV**-1 Vpr binding to **Gag**.

6. The method of claim 5 wherein said binding level is determined by the addition of a labeled antibody.

7. The method of claim 5 wherein said **HIV**-1 Vpr and **Gag** are produced in eukaryotic cells.

8. The method of claim 5 wherein said **HIV**-1 Vpr and **Gag** are produced in insect cells.

9. The method of claim 5 comprising the following steps: (I) contacting, in the presence or absence of a test compound, eukaryotically expressed **HIV**-1 Vpr and **Gag**, wherein said Vpr is attached to a solid support; (ii) washing the mixture of step (I) to remove unbound **Gag** protein; and, (iii) determining the level of binding between **HIV**-1 Vpr and **Gag** through the addition of a **Gag**-specific labeled antibody, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting **HIV**-1 Vpr binding to **Gag**.

10. The method of claim 9 wherein said **HIV**-1 Vpr and **Gag** are produced in insect cells.

11. The method of claim 5 comprising the following steps: (I) contacting, in the presence or absence of a test compound, eukaryotically expressed **HIV**-1 Vpr and **Gag**, wherein said **Gag** is attached to a solid support; (ii) washing the mixture of step (I) to remove unbound Vpr protein; and, (iii) determining the level of binding between **HIV**-1 Vpr and **Gag** through the addition of a Vpr-specific labeled antibody, wherein a reduction in binding in the presence of the test compound is indicative of said compound being capable of inhibiting **HIV**-1 Vpr binding to **Gag**.

12. The method of claim 11 wherein said **HIV**-1 Vpr and **Gag** are produced in insect cells.

L32 ANSWER 14 OF 16 USPATFULL on STN
1998:48226 Mason-Pfizer Monkey Retroviral packaging defective vectors.
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Syngenix Limited, Cambridge, England (non-U.S. corporation)
US 5747307 19980505
WO 9317118 19930902
APPLICATION: US 1994-295737 19940826 (8) <--
WO 1993-GB417 19930301 19940826 PCT 371 date 19940826 PCT 102(e) date<--
PRIORITY: GB 1992-4350 19920228
GB 1992-8489 19920416
GB 1992-19935 19920902
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel vectors are capable of producing MPMV (Mason-Pfizer Monkey Virus) proteins but not of packaging MPMV RNA, and the information about the packaging signal in MPMV and HIV can be used to create MPMV and HIV vectors that are capable of transferring foreign genes, e.g. for use in gene therapy.

CLM What is claimed is:

1. A vector comprising a polynucleotide sequence which encodes MPMV (Mason-Pfizer Monkey Virus) proteins, said polynucleotide sequence comprising an MPMV leader sequence which is a deletion mutant of SEQ ID NO. 2, wherein said MPMV leader sequence has an effective deletion of nucleotides which results in MPMV RNA transcribed from said vector being packaging defective, said deletion occurring between the MPMV primer-binding site at nucleotides 348-365 of SEQ ID NO. 2 and the MPMV 5' major splice donor at nucleotides 475-480 of SEQ ID NO. 2.

2. The vector according to claim 1, wherein the deletion comprises bases 28 to 50 of SEQ ID NO. 1.

3. The vector according to claim 1, wherein the deletion comprises bases 51 to 112 of SEQ ID NO. 1.

4. The vector according to claim 1, wherein the deletion comprises SEQ ID NO. 1.

5. The vector according to claim 1, which contains sequences corresponding to a promoter region in the MPMV genome or another genome's polyadenylation sequence but does not contain sequences corresponding to a MPMV long terminal repeat sequence.

6. The vector according to claim 1, which permits expression of MPMV proteins upstream of env or of the remaining proteins, and which optionally also includes a **marker**.

7. A vector comprising nucleotides corresponding to the packaging nucleotides of MPMV, a heterologous gene and, flanking the packaging nucleotides and the heterologous gene, sequences corresponding to those within and near the MPMV long terminal repeat sequence sufficient for packaging, reverse transcription and integration of the vector into target cells and expression of the heterologous gene, wherein the packaging nucleotides are selected from the group consisting of the nucleotides between the MPMV primer-binding site at nucleotides 348-365 of SEQ ID NO. 2 and the MPMV 5' major splice donor at nucleotides 475-480 of SEQ ID NO. 2, nucleotides 28 to 50 of SEQ ID NO. 1, nucleotides 51 to 112 of SEQ ID NO. 1, and nucleotides 1 to 121 of SEQ ID NO. 1.

8. The vector according to claim 7, which comprises the first 500 bp of the MPMV **gag** gene.

9. The vector according to claim 7, wherein nucleotides corresponding to the nucleotide sequence numbered 481-493 between the MPMV 5' major splice donor and the MPMV initiation codon of SEQ ID NO. 2 are deleted.

10. A method of gene transfer in vitro, which comprises transfecting virion-producing cells with one or more packaging-deficient MPMV or

11. The method according to claim 10, which additionally comprises culturing the cells and contacting the resultant virions with mammalian cells.

12. The vector, according to claim 7, which comprises a MPMV or **HIV** LTR sequence as a promoter for the heterologous gene.

L32 ANSWER 15 OF 16 USPATFULL on STN
97:117939 Methods and compositions for inhibiting production of replication competent virus.
Klump, Wolfgang M., Del Mar, CA, United States
Jolly, Douglas J., Leucadia, CA, United States
Chiron Corporation, United States (U.S. corporation)
US 5698446 19971216
APPLICATION: US 1994-305699 19940907 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods and compositions for inhibiting the production of replication competent virus. The invention comprises nucleic acid cassettes encoding a non-biologically active inhibitory molecule which are incorporated into packaging cells and recombinant vector constructs. Upon recombination between various vector construct contained within the producer cell, a biologically active molecule is produced which kills the cell, thereby inhibiting production of replication competent virus.
CLM What is claimed is:
1. A vector for directing the expression of a retroviral structural polypeptide, the vector comprising a promoter operably associated with a structural gene construct and a polyadenylation signal, the structural gene construct comprising a nucleic acid molecule coding for the retroviral structural polypeptide and a non-biologically active inhibitory molecule, wherein the vector inhibits production of replication competent retrovirus resulting from recombination events in retroviral packaging or producer cells.

2. The vector according to claim 1 wherein the retroviral structural polypeptide encoded by the structural gene construct is selected from the group consisting of retroviral env and **gag/pol**.

3. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a toxin selected from the group consisting of tetanus, ricin, and diphtheria toxin.

4. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a ribozyme.

5. The vector according to claim 1 wherein the non-biologically active inhibitory molecule is a prodrug activating enzyme.

6. The vector according to claim 1 wherein the nucleic acid molecule of the structural gene construct further comprises a splice site adjacent to be nucleic acid molecule.

7. A recombinant **retroviral vector** comprising; a) an LTR; b) a packaging signal; c) a tRNA binding site; d) a gene of interest; and e) a nucleic acid cassette comprising a nucleic acid molecule encoding a non-biologically active inhibitory molecule which results in a nucleic acid molecule encoding a biologically active inhibitory molecule upon recombination with the vector according to claim 1.

8. The packaging cell comprising the vector according to claim 1.

9. The vector according to claim 2 wherein the promoter is selected from

the group consisting of an RSV promoter, adenovirus MLP, an SV40 promoter, and CMV MIE.

10. The vector according to claim 2 wherein the retroviral structural polypeptide is env derived from a retrovirus selected from the group consisting of MoMLV, 4070A, HTLV-I, HTLV-II, **HIV**, MPMV, SRV-I, HFV, MFV, **SIV**, GALV, BLV, FeLV, and **FIV**.

11. The vector according to claim 2 wherein the retroviral structural polypeptide is env selected from an amphotropic, polytropic or xenotropic retrovirus.

12. The vector according to claim 2 wherein **gag/pol** is derived from a MoMLV retrovirus.

13. The vector according to claim 11 wherein env is derived from a murine retrovirus.

14. The vector according to claim 5 wherein the non-biologically active inhibitory molecule is the prodrug activating enzyme HSVTK.

15. The recombinant vector according to claim 7 which further comprises a selectable **marker**.

16. The recombinant vector according to claim 7 wherein the nucleic acid molecule encoding the non-biologically active inhibitory molecule is contained in an LTR.

17. A producer cell comprising at least one vector encoding retroviral **gag/pol** and env polypeptides and the recombinant **retroviral vector** according to claim 7.

18. The packaging cell according to claim 8 comprising a vector encoding a retroviral **gag/pol** polypeptide.

19. The packaging cell line according to claim 8 wherein said packaging cell is generated from D17 or HT1080 cells.

20. The packaging cell according to claim 18 further comprising another vector encoding a retroviral env polypeptide.

21. The retroviral packaging cell according to claim 18 further comprising another vector encoding a VSV G polypeptide.

22. A retroviral particle comprising a recombinant **retroviral vector** made by the producer cell according to claim 17.

23. A target cell transduced with the retroviral particle according to claim 22.

24. The target cell according to claim 23 that is an animal cell.

25. The target cell according to claim 24 wherein the animal cell is a human cell.

L32 ANSWER 16 OF 16 USPATFULL on STN
97:63912 Viral vectors.
Wong-Staal, Flossie, San Diego, CA, United States
Mamounas, Michael, Solana Beach, CA, United States
Poeschla, Eric M., San Diego, CA, United States
Kraus, Gunter, La Jolla, CA, United States
Leavitt, Mark, La Jolla, CA, United States
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5650309 19970722

DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Vectors are provided which stably transduce cells, rendering the cells resistant to a target virus. The vectors are amplified upon infection of the cell by a target virus, and spread throughout an infected host in response to infection by the target virus.
CLM What is claimed is:

1. A vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

2. The vector of claim 1, wherein said nucleic acid sequences from said first virus further comprise nucleic acid sequences for nucleic acid replication and encapsidation of the vector.

3. The vector of claim 1, wherein the second virus is an **HIV** virus.

4. The vector of claim 1, wherein said cis-active nucleic acid sequences are AAV 5' and 3' ITR regions.

5. The vector of claim 1, wherein the second virus is **HIV**, and wherein the replication defective, rescuable **HIV** genome encodes a non-functional gene selected from the tat, rev, **gag**, **pol**, env, v/f, vpr, nef, and vpu/vpx genes.

6. The vector of claim 1, wherein the second virus is **HIV**, and wherein the replication defective, rescuable **HIV** genome does not encode a gene selected from the tat, rev, **gag**, **pol**, env, vif, vpr, nef, and vpu/vpx genes.

7. The vector of claim 1, wherein said expression control sequence comprises a constitutive promoter.

8. The vector of claim 1, wherein said expression control sequence comprises an inducible promoter.

9. The vector of claim 1, wherein said expression control sequence comprises an inducible promoter activated in response to viral replication of a replication competent virus corresponding to the replication defective portion of the vector.

10. The vector of claim 1, wherein the anti-viral agent is selected to specifically inhibit the replication of the second virus.

11. The vector of claim 1, wherein said second virus encodes an anti-viral agent selected from the group consisting of an antisense nucleic acid, a ribozyme, a decoy nucleic acid, a transdominant gene and a suicide gene.

12. The vector of claim 1, wherein said second virus encodes an anti viral agent selected from the group consisting of an antisense nucleic acid comprising the **HIV** TAR or RRE sequence, a decoy nucleic acid molecule comprising the TAR sequence or the RRE sequence, a hammerhead ribozyme, and a hairpin ribozyme.

13. The vector of claim 1, further comprising a nucleic acid encoding a selectable **marker** operatively linked to an expression control sequence.

14. The vector of claim 1, further comprising a second anti-viral agent operatively linked to an expression control sequence.

15. A mammalian cell transduced with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

16. The mammalian cell of claim 15 wherein the mammalian cell is a hematopoietic stem cell, fetal cord blood cell, T-lymphocyte or monocyte.

17. A method for inhibiting viral replication in a cell in vitro, comprising transducing the cell with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active AAV nucleic acids for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable retroviral genome, and wherein said nucleic acid sequences of said second virus also encodes an anti-viral agent operably linked to an expression control sequence.

18. The method of claim 17, wherein said transduced cell inhibits viral replication by an **HIV** virus.

19. The method of claim 17, wherein the cell includes genes necessary for activating an expression control sequence contained within said vector.

20. The method of claim 17, wherein the cell is a hematopoietic stem cell, fetal cord blood cell, T-lymphocyte or monocyte.

21. A method for making anti-viral agents in a cell in vitro, comprising transducing the cell with a vector comprising biologically active nucleic acid sequences from a first and second virus, wherein said nucleic acid sequences of said first virus comprise cis-active nucleic acids encoding viral sequences for host cell chromosomal integration, said nucleic acid sequences of said second virus comprise a replication defective, rescuable viral genome, and wherein said nucleic acid sequences of said second virus encode an anti-viral agent operably linked to an expression control sequence, wherein the cell includes genes necessary for activating said expression control sequence, and culturing the cell under conditions for expression of the anti-viral agent.

=> d hs
'HS' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'

The following are valid formats:

The default display format is STD.

ABS -------- AB
ALL -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
EXF, ARTU
ALLG ------- ALL plus PAGE.DRAW
BIB -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI,
PRAI, DT, FS, EXNAM, LREP, CLMN, ECL, DRWN, LN.CNT
BIB.EX ----- BIB for original and latest publication
BIBG ------- BIB plus PAGE.DRAW

BROWSE SEE HELP BROWSE OR HELP DISPLAY BROWSE. BROWSE MUST
entered on the same line as DISPLAY, e.g., D BROWSE.
CAS -------- OS, CC, SX, ST, IT
CBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PRAI, DT, FS
DALL ------- ALL, delimited for post-processing
FP --------- PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI, RLI,
PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL,
NCLM, NCLS, EXF, REP, REN, ARTU, EXNAM, LREP,
CLMN, DRWN, AB
FP.EX ------ FP for original and latest publication
FPALL ------ PI, TI, IN, INA, PA, PAA, PAT, PETRM, DCD, AI,
RLI, PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL, NCLM,
NCLS, EXF, REP, REN,ARTU, EXNAM, LREP, CLMN, DRWN, AB,
PARN, SUMM, DRWD, DETD, CLM
FPBIB ------ PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
RLI, PRAI, REP, REN, EXNAM, LREP, CLM, CLMN, DRWN
FHITSTR ---- HIT RN, its text modification, its CA index name, and
its structure diagram
FPG -------- FP plus PAGE.DRAW
GI --------- PN and page image numbers
HIT -------- All fields containing hit terms
HITRN ------ HIT RN and its text modification
HITSTR ----- HIT RN, its text modification, its CA index name, and
its structure diagram
IABS ------- ABS, indented with text labels
IALL ------- ALL, indented with text labels
IALLG ------ IALL plus PAGE.DRAW
IBIB ------- BIB, indented with text labels
IBIB.EX ---- IBIB for original and latest publication
IBIBG ------ IBIB plus PAGE.DRAW
IMAX ------- MAX, indented with text labels
IMAX.EX ---- IMAX for original and latest publication
IND -------- INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
EXF, ARTU, OS, CC, SX, ST, IT
ISTD ------- STD, indented with text labels
KWIC ------- All hit terms plus 20 words on either side
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RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
EXF, ARTU OS, CC, SX, ST, IT
MAX.EX ----- MAX for original and latest publication
OCC -------- List of display fields containing hit terms
SBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
DT, FS, LN.CNT
SCAN ------- AN, TI, NCL, NCLM, NCLS, IC, ICM, ICS (random display
without answer number. SCAN must be entered on the
same line as DISPLAY, e.g., D SCAN)
STD -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
DT, FS, LN.CNT, INCL, INCLM, INCLS, NCL, NCLM, NCLS,
IC, ICM, ICS, EXF (STD is the default)
STD.EX ----- STD for original and latest publication
TRIAL ------ AN, TI, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC,
ICM, ICS

ENTER DISPLAY FORMAT (STD):d his
'D' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'
'HIS' IS NOT A VALID FORMAT FOR FILE 'USPATFULL'

The following are valid formats:

The default display format is STD.

ABS -------- AB
ALL -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,

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                DRWN, FILE, GOVI, HITNR, HITSTR, IABS, IALL, IALLG,
                INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
                EXF, ARTU
  ALLG ------- ALL plus PAGE.DRAW
  BIB -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD, RLI,
                PRAI, DT, FS, EXNAM, LREP, CLMN, ECL, DRWN, LN.CNT
  BIB.EX ----- BIB for original and latest publication
  BIBG ------- BIB plus PAGE.DRAW
  BROWSE ----- See "HELP BROWSE" or "HELP DISPLAY BROWSE".  BROWSE must
                entered on the same line as DISPLAY, e.g., D BROWSE.
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  DALL ------- ALL, delimited for post-processing
  FP --------- PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI, RLI,
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                CLMN, DRWN, AB
  FP.EX ------ FP for original and latest publication
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                RLI, PRAI, IC, ICM, ICS, INCL, INCLM, INCLS, NCL, NCLM,
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                PARN, SUMM, DRWD, DETD, CLM
  FPBIB ------ PI, TI, IN, INA, PA, PAA, PAT, PTERM, DCD, AI,
                RLI, PRAI, REP, REN, EXNAM, LREP, CLM, CLMN, DRWN
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                its structure diagram
  FPG -------- FP plus PAGE.DRAW
  GI --------- PN and page image numbers
  HIT -------- All fields containing hit terms
  HITRN ------ HIT RN and its text modification
  HITSTR ----- HIT RN, its text modification, its CA index name, and
                its structure diagram
  IABS ------- ABS, indented with text labels
  IALL ------- ALL, indented with text labels
  IALLG ------ IALL plus PAGE.DRAW
  IBIB ------- BIB, indented with text labels
  IBIB.EX ---- IBIB for original and latest publication
  IBIBG ------ IBIB plus PAGE.DRAW
  IMAX ------- MAX, indented with text labels
  IMAX.EX ---- IMAX for original and latest publication
  IND -------- INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
                EXF, ARTU, OS, CC, SX, ST, IT
  ISTD ------- STD, indented with text labels
  KWIC ------- All hit terms plus 20 words on either side
  MAX -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, PTERM, DCD,
                RLI, PRAI, DT, FS, REP, REN, EXNAM, LREP, CLMN, ECL,
                DRWN, AB, GOVI, PARN, SUMM, DRWD, DETD, CLM, INCL,
                INCLM, INCLS, NCL, NCLM, NCLS, IC, ICM, ICS,
                EXF, ARTU OS, CC, SX, ST, IT
  MAX.EX ----- MAX for original and latest publication
  OCC -------- List of display fields containing hit terms
  SBIB ------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
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                without answer number.  SCAN must be entered on the
                same line as DISPLAY, e.g., D SCAN)
  STD -------- AN, TI, IN, INA, PA, PAA, PAT, PI, AI, RLI, PRAI,
                DT, FS, LN.CNT, INCL, INCLM, INCLS, NCL, NCLM, NCLS,
                IC, ICM, ICS, EXF (STD is the default)
  STD.EX ----- STD for original and latest publication
  TRIAL ------ AN, TI, INCL, INCLM, INCLS, NCL, NCLM, NCLS, IC,
                ICM, ICS

ENTER DISPLAY FORMAT (STD):ti

L32  ANSWER 1 OF 16  USPATFULL on STN
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(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
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L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

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E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
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L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25 21 S L24 AND AY<2000
L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000
L31 80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32 16 S L31 AND (MARKER?/CLM)

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148 LENTIVIRAL/CLM

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               5 LENTIVIRAL EXPRESSION VECTOR?/CLM
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=> s l30 and (lentiviral expression vector?/ti)
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           3473 EXPRESSION/TI
           4056 VECTOR?/TI
              0 LENTIVIRAL EXPRESSION VECTOR?/TI
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L34            0 L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)

=> s lentiviral expression vector?
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         196055 EXPRESSION
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L35           10 LENTIVIRAL EXPRESSION VECTOR?
                     (LENTIVIRAL(W)EXPRESSION(W)VECTOR?)

=> d l35,cbib,1-10

L35  ANSWER 1 OF 10  USPATFULL on STN
2004:52619 Transgenic organism.
    Radcliffe, Philippa, Oxford, UNITED KINGDOM
    Mitrophanous, Kyriacos, Oxford, UNITED KINGDOM
    Themis, Michael, London, UNITED KINGDOM
    Oxford BioMedica (UK) Limited (non-U.S. corporation)
    US 2004040052 A1 20040226
    APPLICATION: US 2003-421947  A1  20030424 (10)
    PRIORITY: GB 2001-30797  20011221
    GB 2002-1140  20020118
    GB 2002-11409  20020517
    DOCUMENT TYPE: Utility; APPLICATION.

L35  ANSWER 2 OF 10  USPATFULL on STN
2004:50354 Respiratory delivery for gene therapy and lentiviral delivery
    particle.
    Parsons, David, Marino, AUSTRALIA
    Anson, Don, Thebarton, AUSTRALIA
    Limberis, Maria, Rostrevor, AUSTRALIA
    Fuller, Maria, Prospect, AUSTRALIA
    US 2004037780 A1 20040226
    APPLICATION: US 2002-226638  A1  20020823 (10)
    PRIORITY: AU 2001-8942  20011119
    DOCUMENT TYPE: Utility; APPLICATION.

L35  ANSWER 3 OF 10  USPATFULL on STN
2004:24651 Antibiotic-based gene regulation system.
    Fussenegger, Martin, Zurich, SWITZERLAND
    Weber, Wilfried, Zurich, SWITZERLAND
    US 2004018490 A1 20040129
    APPLICATION: US 2001-949470  A1  20010907 (9)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35  ANSWER 4 OF 10  USPATFULL on STN
2003:194507 Methods of using randomized libraries of zinc finger proteins for
    the identification of gene function.
    Case, Casey C., San Mateo, CA, UNITED STATES
    Liu, Qiang, Foster City, CA, UNITED STATES
    Rebar, Edward J., El Cerrito, CA, UNITED STATES
    Wolffe, Alan P., Orinda, CA, UNITED STATES
    Sangamo BioSciences, Inc. (U.S. corporation)
    US 2003134318 A1 20030717
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APPLICATION: US 2003-[illegible] A1 20030[illegible] (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35 ANSWER 5 OF 10 USPATFULL on STN
2003:175213 Transgenic organism.
Radcliffe, Philippa, Oxford, UNITED KINGDOM
Mitrophanous, Kyriacos, Oxford, UNITED KINGDOM
Themis, Michael, London, UNITED KINGDOM
Oxford BioMedica (UK) Limited (non-U.S. corporation)
US 2003121062 A1 20030626
APPLICATION: US 2002-82122 A1 20020226 (10)
PRIORITY: GB 2001-30797 20011221
GB 2002-1140 20020118
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35 ANSWER 6 OF 10 USPATFULL on STN
2002:265815 Methods of using randomized libraries of zinc finger proteins for the identification of gene function.
Case, Casey C., San Mateo, CA, UNITED STATES
Liu, Qiang, Foster City, CA, UNITED STATES
Rebar, Edward J., El Cerrito, CA, UNITED STATES
Wolffe, Alan P., Orinda, CA, UNITED STATES
US 2002146691 A1 20021010
APPLICATION: US 2000-731558 A1 20001206 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35 ANSWER 7 OF 10 USPATFULL on STN
2002:198257 Methods for therapy of neurodegenerative disease of the brain.
Tuszynski, Mark H., La Jolla, CA, UNITED STATES
Regents of the University of California (U.S. corporation)
US 2002106350 A1 20020808
APPLICATION: US 2001-32952 A1 20011026 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35 ANSWER 8 OF 10 USPATFULL on STN
2002:60559 Vector.
Mitrophanous, Kyriacos A., Oxford, UNITED KINGDOM
Uden, Mark, London, UNITED KINGDOM
Rohll, Jonathan, Reading, UNITED KINGDOM
Kingsman, Susan Mary, Appleton, UNITED KINGDOM
Kingsman, Alan John, Appleton, UNITED KINGDOM
US 2002034393 A1 20020321
APPLICATION: US 2001-860996 A1 20010518 (9)
PRIORITY: GB 1998-25524 19981120
DOCUMENT TYPE: Utility; APPLICATION.

L35 ANSWER 9 OF 10 USPATFULL on STN
2001:214887 Lentiviral vectors derived from SIVagm, methods for their preparation and their use for gene transfer into mammalian cells.
Cichutek, Klaus, Frankfurt am Main, Germany, Federal Republic of
Bundesrepublik Deutschland letztvertreten durch den Prasidenten des Paul-Elrich-Instituts Prof. Dr. R. Kruth, Germany, Federal Republic of (non-U.S. corporation)
US 6323031 B1 20011127
APPLICATION: US 2000-517921 20000303 (9)
PRIORITY: DE 1999-19909769 19990305
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L35 ANSWER 10 OF 10 USPATFULL on STN
2001:145067 Lentiviral vectors derived from SIVagm, methods for their preparation and their use for gene transfer into mammalian cells.

Stuttgart, Rhld, Langen, Germany, Federal Republic of
US 2001018202 A1 20010830
APPLICATION: US 2000-734384 A1 20001211 (9)
PRIORITY: DE 1999-19909769 19990305
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

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FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
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E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
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E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
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L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25 21 S L24 AND AY<2000
L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000

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L32          16 S L31 AND (MARKER?/CLM)
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L35          10 S LENTIVIRAL EXPRESSION VECTOR?

=> s l30 and (lentivir? vector?/clm)
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         48267 VECTOR?/CLM
           117 LENTIVIR? VECTOR?/CLM
                 ((LENTIVIR?(W)VECTOR?)/CLM)
L36          15 L30 AND (LENTIVIR? VECTOR?/CLM)

=> d l36,cbib,1-15

L36  ANSWER 1 OF 15  USPATFULL on STN
2003:209962 Expression of HIV polypeptides and production of virus-like
    particles.
    Barnett, Susan W., San Francisco, CA, United States
    Megede, Jan zur, San Francisco, CA, United States
    Greer, Catherine, Oakland, CA, United States
    Selby, Mark, San Francisco, CA, United States
    Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
    US 6602705 B1 20030805
    APPLICATION: US 1999-475515  19991230 (9)                    <--
    PRIORITY: US 1998-114495P  19981231 (60)
    US 1999-168471P  19991201 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36  ANSWER 2 OF 15  USPATFULL on STN
2003:89278 Suppressor of cytokine signaling (SOCS)-3 promoter and methods for
    its use in genetic therapy in humans.
    Auernhammer, Christoph J., Munchen-Pasing, GERMANY, FEDERAL REPUBLIC OF
    Melmed, Shlomo, Los Angeles, CA, United States
    Cedars-Sinai Medical Center, Los Angeles, CA, United States (U.S.
    corporation)
    US 6541244 B1 20030401
    APPLICATION: US 1999-327138  19990607 (9)                    <--
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36  ANSWER 3 OF 15  USPATFULL on STN
2003:89257 Therapeutic Gene.
    Kingsman, Alan John, Greystones, Middle Street, Islip, Oxon OX5 2SF, UNITED
    KINGDOM
    Kingsman, Susan Mary, Greystones, Middle Street, Islip, Oxon OX5 2SF,
    UNITED KINGDOM
    US 6541219 B1 20030401
    WO 9818934  19980507
    APPLICATION: US 1999-254832  19990621 (9)                    <--
    WO 1997-GB2969 19971028                                      <--
    PRIORITY: GB 1996-22500  19961029
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36  ANSWER 4 OF 15  USPATFULL on STN
2002:228311 LENTIVIRUS BASED VECTOR AND VECTOR SYSTEM.
    UBERLA, KLAUS, MOEHRENDORF, GERMANY, FEDERAL REPUBLIC OF
    US 2002123471 A1 20020905
    APPLICATION: US 1999-380323  A1  19991122 (9)                <--
    WO 1998-EP1191 19980303                                      <--
    PRIORITY: DK 1997-238  19970603
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

L36 ANSWER 5 OF 15 USPATFULL on STN
2002:217078 Retroviral hybrid vectors pseudotyped with LCMV.
Von Laer, Meike-Dorothee, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Beyer, Winfried, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Heinrich-Pette-Institut, Hamburg, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
US 6440730 B1 20020827
APPLICATION: US 1999-309572 19990511 (9) <--
PRIORITY: DE 1998-19856463 19981126
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 6 OF 15 USPATFULL on STN
2002:140843 GENE TRANSFER TO PANCREATIC B CELLS FOR PREVENTION OF ISLET DYSFUNCTION.
GIANNOUKAKIS, NICK, PITTSBURGH, PA, UNITED STATES
ROBBINS, PAUL D., MY. LEBANON, PA, UNITED STATES
TRUCCO, MASSIMO, PITTSBURGH, PA, UNITED STATES
US 2002071824 A1 20020613
APPLICATION: US 1999-320767 A1 19990527 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 7 OF 15 USPATFULL on STN
2001:208871 Method of controlling L-Dopa production and of treating dopamine deficiency.
Mandel, Ronald J., Lund, Sweden
Leff, Stuart E., Alanta, GA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6319905 B1 20011120
APPLICATION: US 1999-314790 19990519 (9) <--
PRIORITY: US 1998-114016P 19981229 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 8 OF 15 USPATFULL on STN
2001:202863 Transfection, storage and transfer of male germ cells for generation of transgenic species and genetic therapies.
Readhead, Carol W., 2185 San Pasqual St., Pasadena, CA, United States 91107
Winston, Robert, 11 Denman Drive, London NW11 6RE, United Kingdom
US 6316692 B1 20011113
APPLICATION: US 1998-191920 19981113 (9) <--
PRIORITY: US 1997-65825P 19971114 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 9 OF 15 USPATFULL on STN
2001:136438 Lentivirus-based gene transfer vectors.
Olsen, John C., Chapel Hill, NC, United States
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
US 6277633 B1 20010821
APPLICATION: US 1998-76707 19980512 (9) <--
PRIORITY: US 1997-46891P 19970513 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 10 OF 15 USPATFULL on STN
2001:109777 USE OF LENTIVIRAL VECTORS FOR ANTIGEN PRESENTATION IN DENDRITIC CELLS.
WONG-STAAL, FLOSSIE, SAN DIEGO, CA, United States
LI, XINGIANG, SAN DIEGO, CA, United States
KAN-MITCHELL, JUNE, RANCHO SANTA FE, CA, United States
THE UNIVERSITY OF CALIFORNIA (U.S. corporation)
US 2001007659 A1 20010712

APPLICATION: US 1998-[illegible] (9)
PRIORITY: US 1997-43264P 19970417 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 11 OF 15 USPATFULL on STN
2001:51844 Inducible genetic suppression of cellular excitability.
Johns, David C., Elkridge, MD, United States
Marban, Eduardo, Lutherville, MD, United States
The Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)
US 6214620 B1 20010410
APPLICATION: US 1999-407945 19990929 (9) <--
PRIORITY: US 1998-102140P 19980929 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 12 OF 15 USPATFULL on STN
2001:29365 Vectors comprising SAR elements.
Agarwal, Manju, Sunnyvale, CA, United States
Plavec, Ivan, Sunnyvale, CA, United States
Veres, Gabor, Palo Alto, CA, United States
Novartis AG, Basel, Switzerland (non-U.S. corporation)
US 6194212 B1 20010227
WO 9746687 19971211
APPLICATION: US 1998-194301 19981123 (9) <--
WO 1997-EP2972 19970606 19981123 PCT 371 date 19981123 PCT 102(e) date<--
PRIORITY: US 1996-19231P 19960606 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 13 OF 15 USPATFULL on STN
2000:164272 Oligmers which inhibit expression of collagen genes.
Guntaka, Ramareddy V., 2909 Bluffcreek Dr., Columbia, MO, United States 65201
Weber, Karl T., Columbia, MO, United States
Kovacs, Attilla, St. Louis, MO, United States
Kandala, Jagannadhachari, Columbia, MO, United States
Guntaka, Ramareddy V., Columbia, MO, United States (U.S. individual)
US 6156513 20001205
APPLICATION: US 1998-130888 19980807 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 14 OF 15 USPATFULL on STN
2000:149940 Expression vectors and methods of use.
Marasco, Wayne A., Wellesley, MA, United States
Richardson, Jennifer, Boston, MA, United States
Parolin, Maria Cristina, Padua, Italy
Sodroski, Joseph G., Medford, MA, United States
Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)
US 6143520 20001107
APPLICATION: US 1998-60659 19980415 (9) <--
PRIORITY: US 1995-5359P 19951016 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 15 OF 15 USPATFULL on STN
2000:4679 Vector and method of use for nucleic acid delivery to non-dividing cells.
Verma, Inder, Solana Beach, CA, United States
Trono, Didier, San Diego, CA, United States
Naldini, Luigi, Del Mar, CA, United States
Gallay, Philippe, Solana Beach, CA, United States
The Salk Institute for Biological Studies, La Jolla, CA, United States

(US Corporation)
US 6013516 20000111
APPLICATION: US 1995-540259 19951006 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
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E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21 13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25 21 S L24 AND AY<2000
L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000
L31 80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)

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L33           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
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L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)

=> s l36 and marker?/clm
         18328 MARKER?/CLM
L37          3 L36 AND MARKER?/CLM

=> d l37,cbib,1-3

L37  ANSWER 1 OF 3  USPATFULL on STN
2002:217078 Retroviral hybrid vectors pseudotyped with LCMV.
    Von Laer, Meike-Dorothee, Hamburg, GERMANY, FEDERAL REPUBLIC OF
    Beyer, Winfried, Hamburg, GERMANY, FEDERAL REPUBLIC OF
    Heinrich-Pette-Institut, Hamburg, GERMANY, FEDERAL REPUBLIC OF (non-U.S.
    corporation)
    US 6440730 B1 20020827
    APPLICATION: US 1999-309572  19990511 (9)                          <--
    PRIORITY: DE 1998-19856463  19981126
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L37  ANSWER 2 OF 3  USPATFULL on STN
2001:202863 Transfection, storage and transfer of male germ cells for
    generation of transgenic species and genetic therapies.
    Readhead, Carol W., 2185 San Pasqual St., Pasadena, CA, United States
    91107
    Winston, Robert, 11 Denman Drive, London NW11 6RE, United Kingdom
    US 6316692 B1 20011113
    APPLICATION: US 1998-191920  19981113 (9)                          <--
    PRIORITY: US 1997-65825P  19971114 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L37  ANSWER 3 OF 3  USPATFULL on STN
2000:149940 Expression vectors and methods of use.
    Marasco, Wayne A., Wellesley, MA, United States
    Richardson, Jennifer, Boston, MA, United States
    Parolin, Maria Cristina, Padua, Italy
    Sodroski, Joseph G., Medford, MA, United States
    Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S.
    corporation)
    US 6143520 20001107
    APPLICATION: US 1998-60659  19980415 (9)                           <--
    PRIORITY: US 1995-5359P  19951016 (60)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.


=> d l37,cbib,ab,clm,3

L37  ANSWER 3 OF 3  USPATFULL on STN
2000:149940 Expression vectors and methods of use.
    Marasco, Wayne A., Wellesley, MA, United States
    Richardson, Jennifer, Boston, MA, United States
    Parolin, Maria Cristina, Padua, Italy
    Sodroski, Joseph G., Medford, MA, United States
    Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S.
    corporation)
    US 6143520 20001107
    APPLICATION: US 1998-60659  19980415 (9)                           <--
    PRIORITY: US 1995-5359P  19951016 (60)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

AB The present invention is related to vectors and methods for increasing the expression of a desired gene product. Preferably this invention is used with genes expressing proteins that are not well tolerated by mammalian cells or where high levels of expression are necessary. In certain preferred embodiments it can be used as part of a multi-tiered expression system and with methods of intracellularly targeting a molecule.

CLM What is claimed is:

1. A **lentiviral vector** containing a gene of interest operably linked to a selectable **marker** gene by an internal ribosome entry site (IRES).

2. The **lentiviral vector** of claim 1, wherein the gene of interest is a gene whose expression in a mammalian cell is selected against as determined by comparing a cell transduced using a divalent vector or co-transfection with a selectable **marker** and said gene, with a control cell transduced using a divalent vector or co-transfection with only said selectable **marker**.

3. The **lentiviral vector** of claim 2, wherein the gene of interest is selected from the group consisting of a gene for HTLV-1 tax, HTLV-2 tax, an antibody and a protein that is part of a multi-tiered expression system.

4. The **lentiviral vector** of claim 1, wherein a defective **lentiviral vector** is used.

5. The **lentiviral vector** of claim 4, wherein the defective **lentiviral vector** is a **lentiviral vector** containing multiple splice donor and splice acceptor sites.

6. The **lentiviral vector** of claim 5, wherein the **lentiviral vector** is an **HIV** viral vector.

7. A method of using the vector of claim 1 to obtain forced expression of the gene of interest which comprises using the vector of claim 1 to transduce a mammalian cell, culturing the transduced cell under conditions sufficed to express the selectable **marker** gene, and then exerting selection pressure on the transduced cell to select for that selectable **marker**.

8. The **lentiviral vector** of claim 3, wherein the gene of interest is an antibody gene.

9. The **lentiviral vector** containing two different genes linked together by an internal ribosome entry site (IRES).

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3

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                E FISCHBACH M A/AU
L7          134 S E2
L8            2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9            2 S L8 NOT L6
                E KUHN R J/AU
L10          95 S E3
L11           9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12           8 S L11 NOT (L6 OR L9)
                E JEFFERS S A/AU
L13          33 S E2 OR E5
L14           2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15          10 S E3

     FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
                E SANDERS D A/IN
L16           4 S E3
                E FISCHBACH M A/IN
L17           1 S E3
                E KUHN R J/IN
L18           2 S E3
                E JEFFERS S A/IN
L19           3 S E3
                E NORTH C L/IN
L20           1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21       13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22        2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23         106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24          28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25          21 S L24 AND AY<2000
L26          12 S L25 AND (MARKER?/CLM)
L27          10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28         689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29           5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30         283 S L28 AND AY<2000
L31          80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32          16 S L31 AND (MARKER?/CLM)
L33           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

=> d l36,cbib,1-15

L36  ANSWER 1 OF 15  USPATFULL on STN
2003:209962 Expression of HIV polypeptides and production of virus-like
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    Barnett, Susan W., San Francisco, CA, United States
    Megede, Jan zur, San Francisco, CA, United States
    Greer, Catherine, Oakland, CA, United States
    Selby, Mark, San Francisco, CA, United States
    Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
    US 6602705 B1 20030805
    APPLICATION: US 1999-475515  19991230 (9)                          <--
    PRIORITY: US 1998-114495P  19981231 (60)
    US 1999-168471P  19991201 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36  ANSWER 2 OF 15  USPATFULL on STN
```

2003:89257 Suppressor of cytokine signaling (SOCS) 3 promoter and methods for its use in genetic therapy in humans.
Auernhammer, Christoph J., Munchen-Pasing, GERMANY, FEDERAL REPUBLIC OF
Melmed, Shlomo, Los Angeles, CA, United States
Cedars-Sinai Medical Center, Los Angeles, CA, United States (U.S. corporation)
US 6541244 B1 20030401
APPLICATION: US 1999-327138 19990607 (9) <--
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 3 OF 15 USPATFULL on STN
2003:89257 Therapeutic Gene.
Kingsman, Alan John, Greystones, Middle Street, Islip, Oxon OX5 2SF, UNITED KINGDOM
Kingsman, Susan Mary, Greystones, Middle Street, Islip, Oxon OX5 2SF, UNITED KINGDOM
US 6541219 B1 20030401
WO 9818934 19980507
APPLICATION: US 1999-254832 19990621 (9) <--
WO 1997-GB2969 19971028 <--
PRIORITY: GB 1996-22500 19961029
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 4 OF 15 USPATFULL on STN
2002:228311 LENTIVIRUS BASED VECTOR AND VECTOR SYSTEM.
UBERLA, KLAUS, MOEHRENDORF, GERMANY, FEDERAL REPUBLIC OF
US 2002123471 A1 20020905
APPLICATION: US 1999-380323 A1 19991122 (9) <--
WO 1998-EP1191 19980303 <--
PRIORITY: DK 1997-238 19970603
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 5 OF 15 USPATFULL on STN
2002:217078 Retroviral hybrid vectors pseudotyped with LCMV.
Von Laer, Meike-Dorothee, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Beyer, Winfried, Hamburg, GERMANY, FEDERAL REPUBLIC OF
Heinrich-Pette-Institut, Hamburg, GERMANY, FEDERAL REPUBLIC OF (non-U.S. corporation)
US 6440730 B1 20020827
APPLICATION: US 1999-309572 19990511 (9) <--
PRIORITY: DE 1998-19856463 19981126
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 6 OF 15 USPATFULL on STN
2002:140843 GENE TRANSFER TO PANCREATIC B CELLS FOR PREVENTION OF ISLET DYSFUNCTION.
GIANNOUKAKIS, NICK, PITTSBURGH, PA, UNITED STATES
ROBBINS, PAUL D., MY. LEBANON, PA, UNITED STATES
TRUCCO, MASSIMO, PITTSBURGH, PA, UNITED STATES
US 2002071824 A1 20020613
APPLICATION: US 1999-320767 A1 19990527 (9) <--
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 7 OF 15 USPATFULL on STN
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Mandel, Ronald J., Lund, Sweden
Leff, Stuart E., Alanta, GA, United States
Cell Genesys, Inc., Foster City, CA, United States (U.S. corporation)
US 6319905 B1 20011120
APPLICATION: US 1999-314790 19990519 (9) <--

PRIORITY: [illegible]
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 8 OF 15 USPATFULL on STN
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Readhead, Carol W., 2185 San Pasqual St., Pasadena, CA, United States 91107
Winston, Robert, 11 Denman Drive, London NW11 6RE, United Kingdom
US 6316692 B1 20011113
APPLICATION: US 1998-191920 19981113 (9) <--
PRIORITY: US 1997-65825P 19971114 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 9 OF 15 USPATFULL on STN
2001:136438 Lentivirus-based gene transfer vectors.
Olsen, John C., Chapel Hill, NC, United States
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
US 6277633 B1 20010821
APPLICATION: US 1998-76707 19980512 (9) <--
PRIORITY: US 1997-46891P 19970513 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 10 OF 15 USPATFULL on STN
2001:109777 USE OF LENTIVIRAL VECTORS FOR ANTIGEN PRESENTATION IN DENDRITIC CELLS.
WONG-STAAL, FLOSSIE, SAN DIEGO, CA, United States
LI, XINGIANG, SAN DIEGO, CA, United States
KAN-MITCHELL, JUNE, RANCHO SANTA FE, CA, United States
THE UNIVERSITY OF CALIFORNIA (U.S. corporation)
US 2001007659 A1 20010712
APPLICATION: US 1998-61986 A1 19980417 (9) <--
PRIORITY: US 1997-43264P 19970417 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 11 OF 15 USPATFULL on STN
2001:51844 Inducible genetic suppression of cellular excitability.
Johns, David C., Elkridge, MD, United States
Marban, Eduardo, Lutherville, MD, United States
The Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)
US 6214620 B1 20010410
APPLICATION: US 1999-407945 19990929 (9) <--
PRIORITY: US 1998-102140P 19980929 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 12 OF 15 USPATFULL on STN
2001:29365 Vectors comprising SAR elements.
Agarwal, Manju, Sunnyvale, CA, United States
Plavec, Ivan, Sunnyvale, CA, United States
Veres, Gabor, Palo Alto, CA, United States
Novartis AG, Basel, Switzerland (non-U.S. corporation)
US 6194212 B1 20010227
WO 9746687 19971211
APPLICATION: US 1998-194301 19981123 (9) <--
WO 1997-EP2972 19970606 19981123 PCT 371 date 19981123 PCT 102(e) date<--
PRIORITY: US 1996-19231P 19960606 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 13 OF 15 USPATFULL on STN
2000:164272 Oligmers which inhibit expression of collagen genes.
Guntaka, Ramareddy V., 2909 Bluffcreek Dr., Columbia, MO, United States 65201
Weber, Karl T., Columbia, MO, United States
Kovacs, Attilla, St. Louis, MO, United States
Kandala, Jagannadhachari, Columbia, MO, United States
Guntaka, Ramareddy V., Columbia, MO, United States (U.S. individual)
US 6156513 20001205
APPLICATION: US 1998-130888 19980807 (9) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 14 OF 15 USPATFULL on STN
2000:149940 Expression vectors and methods of use.
Marasco, Wayne A., Wellesley, MA, United States
Richardson, Jennifer, Boston, MA, United States
Parolin, Maria Cristina, Padua, Italy
Sodroski, Joseph G., Medford, MA, United States
Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)
US 6143520 20001107
APPLICATION: US 1998-60659 19980415 (9) <--
PRIORITY: US 1995-5359P 19951016 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L36 ANSWER 15 OF 15 USPATFULL on STN
2000:4679 Vector and method of use for nucleic acid delivery to non-dividing cells.
Verma, Inder, Solana Beach, CA, United States
Trono, Didier, San Diego, CA, United States
Naldini, Luigi, Del Mar, CA, United States
Gallay, Philippe, Solana Beach, CA, United States
The Salk Institute for Biological Studies, La Jolla, CA, United States (U.S. corporation)
US 6013516 20000111
APPLICATION: US 1995-540259 19951006 (8) <--
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l36,cbib,ab,clm,4,9,10,14,15

L36 ANSWER 4 OF 15 USPATFULL on STN
2002:228311 LENTIVIRUS BASED VECTOR AND VECTOR SYSTEM.
UBERLA, KLAUS, MOEHRENDORF, GERMANY, FEDERAL REPUBLIC OF
US 2002123471 A1 20020905
APPLICATION: US 1999-380323 A1 19991122 (9) <--
WO 1998-EP1191 19980303 <--
PRIORITY: DK 1997-238 19970603
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to **retroviral vectors** which will infect and confer efficient gene transfer to non-dividing cells including the cells of the central nervous system. The vector system of the present invention is useful as a gene transfer vehicle for gene therapy, i.e. of the central nervous system.
CLM What is claimed is:
1. A **lentivirus** based vector comprising all or parts of the left and right hand LTR sequences, wherein the gng, pol and env coding sequences have all been partially or fully deleted or mutated and wherein one or more or all of the sequences coding for vif, vpr, vpx, and nef have independently or in combination wholly or partially been deleted, but where optionally the tat and rev genes are still expressed, and wherein the nuclear localisation signal and/or the C-terminal coding sequence of

the matrix protein have specifically been deleted or mutated.

2. The retroviral **lentivirus vector** according to claim 1, comprising a gene relevant for the treatment of a central nervous system disease or disorder, including such genes such as the NGF (nerve growth factor) gene, the GDNF (glia derived neurotrophic factor) gene, the DAT (dopamine transporter) gene, or the tyrosine hydroxylase gene; or a gene relevant for metabolic liver disease or any other relevant disease.

3. A retroviral **lentivirus** based vector system comprising the **lentivirus vector** according to claim 1 or 2 as a first component, and a packaging cell line that synthesises the Gag and Pol proteins of said **lentivirus** as well as the Env protein of the said **lentivirus** or of a heterologous Env protein, and where optionally the tat and rev genes are also expressed.

4. The retroviral **lentivirus** based vector system according to claim 3, wherein the vector is derived from **HIV** type 1 or 2, **SIV**, **FIV**, **BIV**, CAEV, **EIAV**, while Env is derived from mammalian C-type retroviruses like, amphotropic, polytropic or xenotropic murine leukemia viruses (MLV), murine sarcoma virus, feline leukemia viruses, simian sarcoma viruses, reticuloendotheliosis virus, or spleen necrosis virus; or from Rous sarcoma viruses; or from gibbon ape leukemia viruses; or from Spleen Nekrosis viruses; or from **HIV**, human immunodeficiency virus 1 and 2; or from **SIV**, simian immunodeficiency virus; or from B-type viruses like mouse mammary tumor viruses; or from D-type viruses like Mason Pfizer monkey virus or Simian Retroviruses; or from HTLV, human T cell leukemia virus type 1 and 2; or from Spumaviruses like, Simiam foamy virus, Human foamy virus, or feline syncytium-forming virus; or from G-protein of vesicular stomatitis virus (VSV-G).

5. The retroviral **lentivirus** based vector system according to claim 3 or 4, wherein the vector is derived from **SIV** and the Env is derived from **SIV** or an amphotropic, polytropic or xenotropic murine leukemia virus or from vesicular stomatitis virus (VSV-G-protein).

6. A retroviral particle comprising a retroviral **lentivirus** based vector according to any of the preceding claims 1 to 5.

7. The retroviral particle according to claim 6 obtainable by transfecting a packaging cell of the **lentivirus** based vector system according to any of the preceding claims 3 to 5 with the **lentivirus** based vector according to any of the preceding claims 1 to 5.

8. A retroviral provirus produced by infection of target cells with the retroviral particle according to claim 6 or 7.

9. mRNA of a retroviral provirus according to claim 8.

10. RNA of the retroviral **lentivirus** based vector according to any of the preceding claims 1 to 5.

11. cDNA of the RNA according to claim 10.

12. A host cell infected with the retroviral particle according to claim 6 or 7.

13. The retroviral particle according to claim 6 or 7 and/or the **lentivirus** based vector system according to any of the preceding claims 3 to 5 and/or the **lentivirus** based vector according to any of the preceding claims 1 to 5 for use in the treatment of a central nervous system disease or disorder or a metabolic liver disease or any other relevant disease or disorder.

14. A pharmaceutical composition containing a therapeutically effective amount of the retroviral particle according to claim 6 or 7 and/or the

retroviral **lentivirus** based vector system according to any of the preceding claims 3 to 5.

15. Use of the **lentivirus vector** according to any of the preceding claims 1 to 5 and/or of the retroviral **lentivirus** based vector system according to any of the preceding claims 3 to 5 and/or of the retroviral particle according to claim 6 or 7 for producing a pharmaceutical composition for gene therapy.

16. The use according to claim 15 for the treatment of a central nervous system disease or disorder or a metabolic liver disease or any other relevant disease or disorder.

17. A method for introducing homologous and/or heterologous nucleotide sequences into target cells comprising infecting the target cells with retroviral particles according to claim 6 or 7.

18. A method of treating a central nervous system disorder or disease or metabolic liver disease or any other relevant disease or disorder of an animal including a human, which method comprises administering to a person in need thereof a therapeutically effective amount of the **retroviral vector** system according to any of the preceding claims 3 to 5 and/or of the retroviral particle according to claim 6 or 7.

19. A method of immunising, by vaccination or therapeutic vaccination, an animal including a human, against **lentivirus** infection, which method comprises administering to a person in need thereof a therapeutically effective amount of the **retroviral vector** system according to any of the preceding claims 3 to 5 and/or of the retroviral particle according to claim 6 or 7.

20. The method of claim 19 wherein the **lentivirus** infection is **HIV** or **SIV** or HTLV.

L36 ANSWER 9 OF 15 USPATFULL on STN
2001:136438 Lentivirus-based gene transfer vectors.
Olsen, John C., Chapel Hill, NC, United States
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)
US 6277633 B1 20010821
APPLICATION: US 1998-76707 19980512 (9) <--
PRIORITY: US 1997-46891P 19970513 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A recombinant lentiviral vector expression system comprises a first vector that comprises a nucleic acid sequence of at least part of the Equine Infectious Anemia Virus (EIAV) genome. The vector contains at least one defect in at least one gene encoding an EIAV structural protein, but is preferably a gaglpol expression vector. The expression system further comprises a second vector, also comprising a nucleic acid sequence of at least part of the Equine Infectious Anemia Virus (EIAV) genome, and additionally containing a multiple cloning site wherein a heterologous gene may be inserted. The expression system also comprises a third vector which expresses a viral envelope protein. The first and third vectors are packaging signal-defective. When the expression system is transfected into a lentivirus-permissive cell, replication-defective EIAV particles may be produced, which particles are useful in delivering heterologous genes to a broad range of both dividing and non-dividing cells.
CLM What is claimed is:
1. A recombinant **lentiviral vector** expression system comprising: (a) a first vector comprising an Equine Infectious Anemia Virus (**EIAV**) nucleic acid sequence encoding **EIAV** gag and **EIAV** pol, wherein said vector (i) comprises at least one defect in at least one gene encoding an **EIAV** structural protein, and (ii) comprises a defective packaging

signal; (b) a second vector comprising an **EIAV** nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome, wherein said vector (i) comprises a competent packaging signal, and (ii) comprises a multiple cloning site wherein a heterologous gene may be inserted; and (c) a third vector comprising a viral nucleic acid sequence, wherein said third vector (i) expresses a viral envelope protein, and (ii) comprises a defective packaging signal.

2. A vector system according to claim 1, wherein said second vector is deficient for expression of at least one **EIAV** structural protein.

3. A vector system according to claim 1, wherein said first vector, said second vector, and said third vector are obtained from cDNA clones of the **EIAV** genome.

4. A vector expression system according to claim 1, wherein said first vector is a gag-pol expression vector, and wherein said vector comprises a defect in the env gene.

5. A vector expression system according to claim 4, wherein said defect in the env gene is a deletion mutation.

6. A vector expression system according to claim 1, wherein said first vector and said second vector each comprise a defect in the env gene.

7. A vector expression system according to claim 1, wherein said third vector encodes an envelope protein that is not an **EIAV** envelope protein.

8. A vector expression system according to claim 1, wherein said third vector expresses the vesicular stomatitis virus G glycoprotein.

9. A vector expression system according to claim 1, wherein said second vector comprises a heterologous gene.

10. A vector expression system according to claim 9, wherein said heterologous gene encodes an antigenic protein or peptide.

11. A vector expression system according to claim 1, wherein said first vector is selected from the group consisting of the plasmid pEV53 and the plasmid pEV53A; said second vector is selected from the group consisting of pEC-lacZ and pEC-puro; and said third vector is the plasmid pCI-VSV-G.

12. The plasmid set forth in FIG. 2A as pEV53.

13. The plasmid set forth in FIG. 2B as pEV53A.

14. The plasmid set forth in FIG. 2 as pEC lacZ.

15. The plasmid set forth in FIG. 3 as pEC-puro.

16. The plasmid set forth in FIG. 4 as pCI-VSV-G.

17. A method of producing a replication-defective **lentivirus** particle, comprising transfecting a cell with: (a) a first vector comprising an Equine Infectious Anemia Virus (**EIAV**) nucleic acid sequence encoding **EIAV** gag and **EIAV** pol, wherein said vector (i) comprises at least one defect in at least one gene encoding an **EIAV** structural protein, and (ii) comprises a defective packaging signal; (b) a second vector comprising an **EIAV** nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome wherein said vector (i) comprises a competent packaging signal, and (ii) comprises a multiple cloning site wherein a heterologous gene may be inserted; and (c) a third vector comprising a nucleic acid sequence of a virus, wherein said third vector (i) expresses a viral

envelope protein, and (2) comprises a defective packaging signal, wherein the cell produces a replication-defective **lentivirus** particle.

18. A method according to claim 17, wherein said cell is a non-dividing cell.

19. A method according to claim 17, wherein said second vector comprises a heterologous gene.

20. A replication-defective **lentivirus** particle produced according to the method of claim 17.

21. A cell comprising a replication-defective **lentiviral** particle, wherein said **lentiviral** particle is produced according to the method of claim 17.

22. An infectious **EIAV** particle comprising a nucleic acid sequence encoding a promoter and a gene sequence heterologous to **EIAV**, and wherein said nucleic acid sequence is defective in encoding at least one **EIAV** structural protein so that said virus particle is replication defective.

23. A method of producing a **lentiviral** stock comprising: (a) transfecting a **lentivirus**-permissive cell with (i) a first vector comprising an Equine Infectious Anemia Virus (**EIAV**) nucleic acid sequence encoding **EIAV** gag and **EIAV** pol, wherein said vector (1) comprises at least one defect in at least one gene encoding an **EIAV** structural protein, and (2) comprises a defective packaging signal; (ii) a second vector comprising an **EIAV** nucleic acid sequence comprising cis-acting sequence elements required for reverse transcription of the vector genome wherein said vector (1) comprises a competent packaging signal, (2) comprises a heterologous gene; and (iii) a third vector comprising a nucleic acid sequence of a virus, wherein said third vector (1) expresses a viral envelope protein, and (2) comprises a defective packaging signal; (b) growing the cell under cell culture conditions sufficient to allow production of replication-defective **lentivirus** particles in the cell; and (c) collecting said replication-defective **lentivirus** particles from the cell.

24. A method according to claim 23, wherein said producer cell is grown in a cell culture medium, and wherein said replication-defective **lentivirus** particles are collected from said medium.

25. A method of making a packaging cell, comprising transfecting a **lentivirus**-permissive cell with a vector comprising an **EIAV** nucleic acid sequence, wherein said vector comprises a defective packaging signal.

26. A method according to claim 25, wherein said vector is a gag-pol expression vector.

27. A method according to claim 25, wherein said vector is selected from the group consisting of the plasmid pEV53 and the plasmid pEV53 A.

28. A method according to claim 25, wherein said **lentivirus**-permissive cell is a human 293 cell.

29. A packaging cell comprising a **lentivirus**-permissive host cell comprising an **EIAV** nucleic acid sequence encoding at least one **EIAV** structural protein, wherein said nucleic acid sequence is packaging-signal defective, such that the cell itself produces at least one **EIAV** structural protein, but does not produce replication-competent infectious virus.

2001:169777 USE OF LENTIVIRAL VECTORS FOR ANTIGEN PRESENTATION IN DENDRITIC CELLS.
WONG-STAAL, FLOSSIE, SAN DIEGO, CA, United States
LI, XINGIANG, SAN DIEGO, CA, United States
KAN-MITCHELL, JUNE, RANCHO SANTA FE, CA, United States
THE UNIVERSITY OF CALIFORNIA (U.S. corporation)
US 2001007659 A1 20010712
APPLICATION: US 1998-61986 A1 19980417 (9) <--
PRIORITY: US 1997-43264P 19970417 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods for inducing immunity in a subject by using dendritic cells transduced with a lentivirus vector constructed to deliver an antigenic epitope. The methods of the invention are particularly suited to inducing immunity to human immunodeficiency virus (HIV) and other viral diseases, as well as to inducing immunity to tumor antigens.

CLM What is claimed is:

1. A method of inducing an immune response in a subject, comprising: administering to the subject, a therapeutically effective amount of a dendritic cell or a progenitor thereof, transduced with a replication defective pseudotyped **lentiviral vector** comprising a nucleic acid sequence encoding an antigen such that the antigen is presented on the surface of the dendritic cell.

2. The method of claim 1, wherein the dendritic cell is an immature dendritic cell.

3. The method of claim 1, wherein the dendritic cell is a non-dividing dendritic cell.

4. The method of claim 1, wherein the progenitor of a dendritic cell is a CD34+ cell.

5. The method of claim 1, wherein the pseudotyped **lentiviral vector** comprises a nucleic acid encoding a cytokine.

6. The method of claim 5, wherein the cytokine is selected from the group consisting of interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), Flt-3/Flk-2 ligand (FL), granulocyte macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF).

7. The method of claim 1, wherein the antigen is a tumor antigen.

8. The method of claim 1, wherein the antigen is a fusion polypeptide comprising more than one antigen.

9. The method of claim 1, wherein the antigen is a **lentiviral** antigen or a fragment thereof.

10. The method of claim 9, wherein the **lentiviral** antigen is a Human Immunodeficiency Virus (**HIV**-1) antigen.

11. The method of claim 9, wherein the **lentiviral** antigen is selected from the group consisting of the gag, pol, env, vpr, vif nef, vpx, tat, rev, vpu gene products and fragments thereof.

12. The method of claim 1, wherein the pseudotyped **lentiviral vector** contains an envelope protein selected from the group consisting of a vesicular stomatitis virus G (VSV-G) protein and a moloney leukemia virus (MLV) protein.

13. The method of claim 1, wherein the pseudotyped **lentiviral vector** is a human immunodeficiency virus (**HIV**-1) vector.

14. The method of claim 1, wherein the pseudotyped **lentiviral vector**

is a non-HIV lentiviral vector.

15. A method of inducing an immune response in a subject, comprising: transducing a dendritic cell or a progenitor of a dendritic cell with a pseudotyped **lentiviral vector** comprising a nucleic acid sequence encoding an antigen such that the antigen is presented on the surface of the dendritic cell to produce a transduced dendritic cell; and contacting the transduced dendritic cells with a T cell to produce an activated T cell, wherein at least one of the pseudotyped **lentiviral vector**, the transduced dendritic cell and the T cell, are administered to the subject.

16. The method of claim 15, wherein the transducing occurs in vivo.

17. The method of claim 15, wherein the transducing occurs in vitro.

18. The method of claim 15, wherein the contacting occurs in vivo.

19. The method of claim 15, wherein the contacting occurs in vitro.

20. The method of claim 15, wherein the dendritic cell is an immature dendritic cell.

21. The method of claim 15, wherein the dendritic cell is non-dividing dendritic cell.

22. The method of claim 15, wherein the progenitor of a dendritic cell is a CD34+ cell.

23. The method of claim 15, wherein the pseudotyped **lentiviral vector** comprises a nucleic acid encoding a cytokine.

24. The method of claim 23, wherein the cytokine is a member selected from group consisting of interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), Flt-3/Flk-2 ligand (FL), granulocyte macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF).

25. The method of claim 15, wherein the antigen is a tumor antigen.

26. The method of claim 15, wherein the antigen is a fusion polypeptide comprising more than one antigen.

27. The method of claim 15, wherein the antigen is a **lentiviral** antigen or a fragment thereof.

28. The method of claim 27, wherein the **lentiviral** antigen is a Human Immunodeficiency Virus (**HIV**-1) antigen.

29. The method of claim 27, wherein the **lentiviral** antigen is selected from the group consisting of the gag, pol, env, vpr, vif nef, vpx, tat, rev, vpu gene products and fragments thereof.

30. The method of claim 15, wherein the pseudotyped **lentiviral vector** contains an envelope protein selected from the group consisting of a vesicular stomatitis virus G (VSV-G) protein and a moloney leukemia virus (MLV) protein.

31. The method of claim 15, wherein the pseudotyped **lentiviral vector** is a human immunodeficiency virus (**HIV**-1) vector.

32. The method of claim 15, wherein the pseudotyped **lentiviral vector** is a non-**HIV lentiviral vector**.

33. A method of activating a T cell comprising contacting a T cell with a dendritic cell having an antigen on its surface, wherein the dendritic cell comprises a pseudotyped **lentiviral vector** comprising a nucleic

acid sequence encoding the antigen, wherein the contacting results in activating the T cell.

34. The method of claim 33, wherein the dendritic cell is an immature dendritic cell.

35. The method of claim 33, wherein the dendritic cell is a non-dividing dendritic cell.

36. The method of claim 33, wherein the progenitor of a dendritic cell is a CD34+cell.

37. The method of claim 33, wherein the activating occurs in vivo.

38. The method of claim 33, wherein the activating occurs in vitro.

39. The method of claim 33, wherein the pseudotyped **lentiviral vector** comprises a nucleic acid encoding a cytokine.

40. The method of claim 39, wherein the cytokine is selected from the group consisting of interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), Flt-3/Flk-2 ligand (FL), granulocyte macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF).

41. The method of claim 33, wherein the antigen is a tumor antigen.

42. The method of claim 33, wherein the antigen is a ftision polypeptide comprising more than one antigen.

43. The method of claim 33, wherein the antigen is a **lentiviral** antigen or a fragment thereof.

44. The method of claim 43. wherein the **lentiviral** antigen is a Human Immunodeficiency Virus (**HIV**-1) antigen.

45. The method of claim 43, wherein the **lentiviral** antigen is selected from the group consisting of the gag, pol, env, vpr, vif, nef, vpx, tat, rev, vpu gene products and fragments thereof.

46. The method of claim 33, wherein the pseudotyped **lentiviral vector** contains an envelope protein selected from the group consisting of a vesicular stomatitis virus G (VSV-G) protein and a moloney leukemia virus (MLV) protein.

47. The method of claim 33, wherein the pseudotyped **lentiviral vector** is a human immunodeficiency virus (**HIV**-1) vector.

48. The method of claim 33, wherein the pseudotyped **lentiviral vector** is a non-**HIV lentiviral vector**.

L36 ANSWER 14 OF 15 USPATFULL on STN
2000:149940 Expression vectors and methods of use.
Marasco, Wayne A., Wellesley, MA, United States
Richardson, Jennifer, Boston, MA, United States
Parolin, Maria Cristina, Padua, Italy
Sodroski, Joseph G., Medford, MA, United States
Dana-Farber Cancer Institute, Inc., Boston, MA, United States (U.S. corporation)
US 6143520 20001107
APPLICATION: US 1998-60659 19980415 (9) <--
PRIORITY: US 1995-5359P 19951016 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is related to vectors and methods for increasing the expression of a desired gene product. Preferably this invention is

used with genes expressing proteins that are not well tolerated by mammalian cells or where high levels of expression are necessary. In certain preferred embodiments it can be used as part of a multi-tiered expression system and with methods of intracellularly targeting a molecule.

CLM What is claimed is:

1. A **lentiviral vector** containing a gene of interest operably linked to a selectable marker gene by an internal ribosome entry site (IRES).

2. The **lentiviral vector** of claim 1, wherein the gene of interest is a gene whose expression in a mammalian cell is selected against as determined by comparing a cell transduced using a divalent vector or co-transfection with a selectable marker and said gene, with a control cell transduced using a divalent vector or co-transfection with only said selectable marker.

3. The **lentiviral vector** of claim 2, wherein the gene of interest is selected from the group consisting of a gene for HTLV-1 tax, HTLV-2 tax, an antibody and a protein that is part of a multi-tiered expression system.

4. The **lentiviral vector** of claim 1, wherein a defective **lentiviral vector** is used.

5. The **lentiviral vector** of claim 4, wherein the defective **lentiviral vector** is a **lentiviral vector** containing multiple splice donor and splice acceptor sites.

6. The **lentiviral vector** of claim 5, wherein the **lentiviral vector** is an **HIV** viral vector.

7. A method of using the vector of claim 1 to obtain forced expression of the gene of interest which comprises using the vector of claim 1 to transduce a mammalian cell, culturing the transduced cell under conditions sufficed to express the selectable marker gene, and then exerting selection pressure on the transduced cell to select for that selectable marker.

8. The **lentiviral vector** of claim 3, wherein the gene of interest is an antibody gene.

9. The **lentiviral vector** containing two different genes linked together by an internal ribosome entry site (IRES).

L36 ANSWER 15 OF 15 USPATFULL on STN

2000:4679 Vector and method of use for nucleic acid delivery to non-dividing cells.

Verma, Inder, Solana Beach, CA, United States
Trono, Didier, San Diego, CA, United States
Naldini, Luigi, Del Mar, CA, United States
Gallay, Philippe, Solana Beach, CA, United States
The Salk Institute for Biological Studies, La Jolla, CA, United States (U.S. corporation)
US 6013516 20000111
APPLICATION: US 1995-540259 19951006 (8) <--
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A recombinant retrovirus capable of infecting a non-dividing cell and a method of producing such a virus is provided. The recombinant retrovirus is preferably of lentivirus origin and is useful for the treatment of a variety of disorders including neurological disorders and disorders of other non-dividing cells.

CLM What is claimed is:

1. A method of producing a **lentivirus** which infects non-dividing cells, said method comprising: a) transfecting a suitable packaging host

cell with the following vectors: a first vector providing a nucleic acid encoding a **lentiviral** gag and a **lentiviral** pol wherein the gag and pol nucleic acid sequences are operably linked to a heterologous regulatory nucleic acid sequence and wherein the vector is defective for nucleic acid sequence encoding functional ENV protein and wherein the nucleic acid of the first vector is devoid of **lentiviral** sequences both upstream and downstream from a splice donor site to a gag initiation site of a **lentiviral** genome; a second vector providing a nucleic acid encoding a non-**lentiviral** ENV protein; a third vector providing a nucleic acid sequence containing a **lentiviral** packaging signal flanked by **lentiviral** cis-acting nucleic acid sequences for reverse transcription, packaging and integration; a heterologous nucleic acid sequence, operably linked to a regulatory nucleic acid sequence; and a less than full length gag structural gene; and b) recovering the recombinant virus.

2. The method of claim 1, wherein the **lentivirus** is human immunodeficiency virus (**HIV**).

3. The method of claim 1, wherein the first vector further includes at least a nucleic acid sequence operably linked to a promoter, wherein the nucleic acid sequence encodes a protein selected from the group consisting of VPR, VIF, NEF, VPX, TAT, REV, VPU and any combination thereof.

4. The method of claim 1, wherein the transfecting further includes providing a nucleic acid sequence of a fourth vector, wherein the nucleic acid sequence is operably linked to a promoter and encodes a protein selected from the group consisting of VPR, VIF, NEF, VPX, TAT, REV, VPU and any combination thereof.

5. The method of claim 1, wherein the less than a full length gag structural gene has a closed reading frame.

6. Three **lentiviral vectors** wherein a first vector comprises a nucleic acid encoding a **lentiviral** gag and a **lentiviral** pol wherein the gag and pol nucleic acid sequences are operably linked to a heterologous regulatory nucleic acid sequence and wherein the first vector is defective for nucleic acid sequence encoding functional ENV protein and nucleic acid of first the vector is devoid of **lentiviral** sequences both upstream and downstream from a splice donor site to a gag initiation site of a **lentiviral** genome; a second vector providing a nucleic acid encoding a non-**lentiviral** ENV protein; and a third vector providing a nucleic acid sequence containing a **lentiviral** packaging signal flanked by **lentiviral** cis-acting nucleic acid sequences for reverse transcription, packaging and integration; a heterologous nucleic acid sequence operably linked to a regulatory nucleic acid sequence; and a less than full length gag structural gene, wherein the third vector is devoid of one or more accessory genes, wherein the three vectors, when introduced into a host cell, express **lentiviral** proteins to form **lentiviral** virions that are replication defective.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN

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                E NORTH CYNTHIA L/IN

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L5           245 S E2 OR E3
L6             6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
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L7           134 S E2
L8             2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9             2 S L8 NOT L6
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L10           95 S E3
L11            9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12            8 S L11 NOT (L6 OR L9)
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L14            2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15           10 S E3

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                E SANDERS D A/IN
L16            4 S E3
                E FISCHBACH M A/IN
L17            1 S E3
                E KUHN R J/IN
L18            2 S E3
                E JEFFERS S A/IN
L19            3 S E3
                E NORTH C L/IN
L20            1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21        13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22         2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23          106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24           28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25           21 S L24 AND AY<2000
L26           12 S L25 AND (MARKER?/CLM)
L27           10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28          689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29            5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30          283 S L28 AND AY<2000
L31           80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32           16 S L31 AND (MARKER?/CLM)
L33            0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34            0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35           10 S LENTIVIRAL EXPRESSION VECTOR?
L36           15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37            3 S L36 AND MARKER?/CLM

=> file medline
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              240.39     280.32

FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004

 FILE LAST UPDATED: 9 MAR 2004 (20040309/UP).  FILE COVERS 1953 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details.
```

MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and
http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
description of changes.

This file contains CAS Registry Numbers for easy and accurate
substance identification.

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=> s (retrovir? or vector? or retrovir? expression vector? or pseudotyp?)
         31891 RETROVIR?
         99135 VECTOR?
         31891 RETROVIR?
        610567 EXPRESSION
         99135 VECTOR?
           174 RETROVIR? EXPRESSION VECTOR?
                 (RETROVIR?(W)EXPRESSION(W)VECTOR?)
          1062 PSEUDOTYP?
L38      123851 (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
                YP?)

=> s l38 and (MoMLV or moloney murine leukemia virus)
           144 MOMLV
          4376 MOLONEY
        108185 MURINE
        170939 LEUKEMIA
        369440 VIRUS
          3578 MOLONEY MURINE LEUKEMIA VIRUS
                 (MOLONEY(W)MURINE(W)LEUKEMIA(W)VIRUS)
L39       1703 L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)

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          6904 POL
L40         85 L39 AND (GAG AND POL)

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L41          0 L40 AND (MULTIVALENT)

=> d his

     (FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
                E SANDERS DAVID A/IN
L1            2 S E3 OR E4
                E FISCHBACH MICHAEL A/IN
L2            1 S E4
                E KUHN RICHARD J/IN
L3            2 S E3
                E JEFFERS SCOTT A/IN
L4            1 S E3
                E NORTH CYNTHIA L/IN

     FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
                E SANDERS D A/AU
L5          245 S E2 OR E3
L6            6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
                E FISCHBACH M A/AU
L7          134 S E2
L8            2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9            2 S L8 NOT L6
                E KUHN R J/AU
L10          95 S E3
L11           9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12           8 S L11 NOT (L6 OR L9)
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L13          33 S E2 OR E5
L14           2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15          10 S E3

     FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
                E SANDERS D A/IN
L16           4 S E3
                E FISCHBACH M A/IN
L17           1 S E3
                E KUHN R J/IN
L18           2 S E3
                E JEFFERS S A/IN
L19           3 S E3
                E NORTH C L/IN
L20           1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21       13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22        2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23         106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24          28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25          21 S L24 AND AY<2000
L26          12 S L25 AND (MARKER?/CLM)
L27          10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28         689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29           5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30         283 S L28 AND AY<2000
L31          80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32          16 S L31 AND (MARKER?/CLM)
L33           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38      123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39        1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40          85 S L39 AND (GAG AND POL)
L41           0 S L40 AND (MULTIVALENT)

=> s l40 and marker?
        255492 MARKER?
L42           7 L40 AND MARKER?

=> d l42,ti,1-7

L42  ANSWER 1 OF 7     MEDLINE on STN
TI   Novel integrating adenoviral/retroviral hybrid vector for gene therapy.

L42  ANSWER 2 OF 7     MEDLINE on STN
TI   Integrating retroviral cassette extends gene delivery of HSV-1
     expression vectors to dividing cells.

L42  ANSWER 3 OF 7     MEDLINE on STN
TI   Characterization of recombination events leading to the production of an
     ecotropic replication-competent retrovirus in a GP+envAM12-derived
     producer cell line.

L42  ANSWER 4 OF 7     MEDLINE on STN
TI   Functional characterization of adenoviral/retroviral chimeric vectors
     and their use for efficient screening of retroviral producer cell lines.
```

L42 ANSWER 5 OF 7 MEDLINE on STN
TI Construction of new **retroviral** producer cells from adenoviral and **retroviral vectors**.

L42 ANSWER 6 OF 7 MEDLINE on STN
TI A murine cell line producing HTLV-I **pseudotype** virions carrying a selectable **marker** gene.

L42 ANSWER 7 OF 7 MEDLINE on STN
TI A mutant murine leukemia virus with a single missense codon in **pol** is defective in a function affecting integration.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
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L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21 13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)

L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000
L31 80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32 16 S L31 AND (MARKER?/CLM)
L33 0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34 0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35 10 S LENTIVIRAL EXPRESSION VECTOR?
L36 15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37 3 S L36 AND MARKER?/CLM

FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38 123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39 1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40 85 S L39 AND (GAG AND POL)
L41 0 S L40 AND (MULTIVALENT)
L42 7 S L40 AND MARKER?

=> d l42,cbib,ab,1-7

L42 ANSWER 1 OF 7 MEDLINE on STN
2002204754. PubMed ID: 11936973. Novel integrating adenoviral/**retroviral** hybrid **vector** for gene therapy. Murphy Stephen J; Chong Heung; Bell Stephen; Diaz Rosa Maria; Vile Richard G. (Molecular Medicine Program, Guggenheim 1836, Mayo Foundation, 200 First Street SW, Rochester, MN 55905, USA.) Human gene therapy, (2002 Apr 10) 13 (6) 745-60. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB A hybrid adenoviral **vector** system was designed to incorporate an excisable **retroviral** cassette that can be stably integrated into the host cell genome. The **vector** contains the terminal sequences of two **Moloney murine leukemia virus retroviral** long terminal repeats (LTRs), fused to form a junction fragment, and is flanked by two loxP recognition sequences. Cre recombinase-directed excision liberates a circular, double-stranded DNA molecule containing the LTR junction fragment. Despite the natural intermediate for **retroviral** integrase being a linear DNA molecule, we show that, in the presence of Cre and **retroviral Gag** and **Pol**, the excised circle can be integrated into the target cell genome through both specific integrase (Int)-directed mechanisms and by a random integration process. The loxP cassette, carrying in addition a selectable **marker** gene, was incorporated into the E1-deleted region of an adenoviral **vector**. Infection of cells expressing Cre, **Gag**, and **Pol** generated clones that survived long term in drug selection (>3 months). Int-mediated integration was demonstrated in seven of nine clones by sequencing of the integration sites. In addition, the introduction of the loxP cassette into 293 cells coexpressing Cre and Int alone in the absence of other **Gag** and **Pol** proteins was sufficient to catalyze the integration mechanism. These experiments demonstrate that it is possible to generate high-titer adenovirus-mediated delivery of a C-type **retroviral** provirus that can subsequently undergo **retroviral** Int-mediated integration into dividing and nondividing cells.

L42 ANSWER 2 OF 7 MEDLINE on STN
2001470636. PubMed ID: 11515376. Integrating **retroviral** cassette extends gene delivery of HSV-1 expression **vectors** to dividing cells. de Felipe P; Izquierdo M; Wandosell F; Lim F. (Universidad Autonoma de Madrid, Madrid, Spain.) BioTechniques, (2001 Aug) 31 (2) 394-402, 404-5. Journal code: 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB **Retroviral vectors** have long been used in a wide variety of gene transfer applications but have certain drawbacks, such as small cargo size, limited tropism, and low titers. HSV expression **vectors** overcome

these disadvantages, but, because they persist in target cells as nonreplicative episomes, they are not retained in all the progeny of dividing cells. Chimeric HSV/AAV products that can mediate transgene integration in human mitotic cells have been constructed, but, to date, genetic modification of dividing cells in animal models using HSV products has not been possible. Here, we report the construction of hybrid HSV/**retroviral vectors** that exhibit up to 50-fold higher transgene integration efficiency compared to **vectors** containing only HSV-1 components. Efficient integration of a **retroviral** transgene cassette encoding pac in human cells required expression of the **Moloney murine leukemia virus gag-pol** genes, but in murine cells, could also be mediated by endogenous activities, albeit at a lower level. Gene delivery was equally efficient in BHK21, a cell line resistant to **retroviral** infection, and transgene retention and expression were observed to be stable for least one month in Hs683 human glioma cells. These **vectors** have wide applications for the genetic modification of many cell types.

L42 ANSWER 3 OF 7 MEDLINE on STN

2000080984. PubMed ID: 10612671. Characterization of recombination events leading to the production of an ecotropic replication-competent **retrovirus** in a GP+envAM12-derived producer cell line. Garrett E; Miller A R; Goldman J M; Apperley J F; Melo J V. (Department of Haematology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Road, London, W12 ONN, United Kingdom.) Virology, (2000 Jan 5) 266 (1) 170-9. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Replication-competent **retrovirus** (RCR) was identified in a GP+envAM12-derived producer cell, containing the MFG-S-Neo **retroviral vector**, using a **marker** rescue assay. Studies were undertaken to determine the origin and structure of this RCR. Receptor interference assays demonstrated that the virus was **pseudotyped** with an ecotropic envelope. Molecular analysis demonstrated the presence of a **MoMLV** ecotropic env recombinant where the neomycin resistance gene of the MFG-S-Neo **vector** was replaced by **MoMLV** ecotropic env. Additional recombinants linking the **retroviral pol** gene to neo and the neo gene to **MoMLV** env were also identified. A full-length **MoMLV retroviral** genome was detected by nested PCR in the contaminated amphotropic producer cells and in cells infected with its supernatant. Unexpectedly, this was also present in the GP+E86 packaging cells together with a previously undescribed envelope construct possessing a full 5' and 3' LTR, although these cells were consistently negative for the presence of RCR. These anomalies in the GP+E86 packaging cell line result in increased homology with the MFG-S-Neo **vector**, leading to an increased risk for the production of RCR. Our findings point to a need for increased vigilance when using these packaging lines to generate replication-defective **retrovirus**.

L42 ANSWER 4 OF 7 MEDLINE on STN

1999145142. PubMed ID: 10022544. Functional characterization of adenoviral/**retroviral** chimeric **vectors** and their use for efficient screening of **retroviral** producer cell lines. Duisit G; Salvetti A; Moullier P; Cosset F L. (Laboratoire de Therapie Genique, CHU Hotel Dieu, Nantes, France.) Human gene therapy, (1999 Jan 20) 10 (2) 189-200. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB We have generated three different E1-deleted replication-defective adenoviral **vectors** expressing either **Moloney murine leukemia virus** (Mo-MuLV) **Gag-Pol** core particle proteins, gibbon ape leukemia virus (GALV) envelope glycoproteins, or an MuLV-derived **retroviral vector** genome encoding mCD2 antigen, a murine cell surface **marker** easily detectable by flow cytometry. Each of the three **vectors** was first characterized individually by infection of cells providing the complementary **retroviral** function(s) and able to induce the production of **retroviral vectors** with an efficiency similar to or higher than that of FLY stable **retroviral** packaging cells [Cosset, F.-L., Takeuchi,

69, 7430-7436]. In small-scale pilot experiments, TE671 cells simultaneously coinfected with the three adenoviral **vectors** efficiently released helper-free **retroviral vectors** in their supernatant, with titers greater than 10(6) infectious particles per milliliter by end-point titrations. Our results also indicated that in contrast to **retroviral vector**-packageable RNAs, the adenovirus-mediated overexpression of both **Gag-Pol** and Env packaging functions had limited impact on **retroviral** titers. The primary mechanism suspected is the premature intracellular cleavage of the Pr65gag precursor that we found in **gag-pol**-expressing cells, which in turn may impair the normal incorporation of high loads of functional Env. Last, the characterization of the adenoviral/**retroviral** chimeric **vectors** allowed the screening of various primate cells for **retroviral** production and we found that three hepatocyte-derived cell lines were highly efficient in the assembly and release of infectious **retroviral** particles.

L42 ANSWER 5 OF 7 MEDLINE on STN

1999129188. PubMed ID: 9930327. Construction of new **retroviral** producer cells from adenoviral and **retroviral vectors**. Lin X. (Protein Studies Program, Oklahoma Medical Research Foundation, Oklahoma City 73104, USA.) Gene therapy, (1998 Sep) 5 (9) 1251-8. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A combination of adenoviral and **retroviral vectors** was used to construct second generation packaging cells that deliver **marker** genes to target cells. A **vector** based upon **Moloney murine leukemia virus** (**MoMLV**) was used to deliver **marker** genes, and an adenovirus-based delivery system was used to deliver **MoMLV** structural genes (**gag pol** and env) to cultured cells. The procedure transformed the cells into new **retroviral** producer cells, which generate replication-incompetent **retroviral** particles in the culture supernatant for transferring **marker** genes to target cells. The titer of the **retroviral**-containing supernatant generated from the second generation producer cells reached above 10(5) c.f.u./ml, which is comparable to the **MoMLV**-based producer cell lines currently used in human gene therapy trials. These observations suggest that this new gene transfer scheme is technically feasible. The **vector** and procedures may be adapted for experimental human gene therapy in which the new producer cells are transplanted into patients for continuous gene transfer.

L42 ANSWER 6 OF 7 MEDLINE on STN

91082439. PubMed ID: 1845835. A murine cell line producing HTLV-I **pseudotype** virions carrying a selectable **marker** gene. Vile R G; Schulz T F; Danos O F; Collins M K; Weiss R A. (Chester Beatty Laboratories, Institute of Cancer Research, London, United Kingdom.) Virology, (1991 Jan) 180 (1) 420-4. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB A murine cell line, EH, expressing the **gag** and **pol** proteins of **Moloney murine leukemia virus** (Mo-MLV) as well as an Mo-MLV recombinant genome with a selectable **marker** (histidinol dehydrogenase), was transfected with a plasmid coding for the gene of the human T-cell leukemia virus type I (HTLV-I) envelope precursor (gp62), placed under the control of the human cytomegalovirus immediate early promoter. One clone, T. 14, was recovered, in which gp62 RNA and protein were detected. Supernatant from this clone transferred the HisD gene to a panel of cell lines which express receptors for HTLV-I, but was unable to pass the **marker** gene to cells which do not express receptors. The colony-forming units were sensitive to HTLV-I receptor interference and to specific neutralization by anti-HTLV-I serum. These data show that hybrid virions were produced in which the envelope proteins of HTLV-I had **pseudotyped** Mo-MLV capside particles containing a selectable recombinant viral genome.

L42 ANSWER 7 OF 7 MEDLINE on STN

85038535. PubMed ID: 6208550. A mutant murine leukemia virus with a single missense codon in **pol** is defective in a function affecting integration. Donehower L A; Varmus H E. Proceedings of the National Academy of Sciences

7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have used site-directed mutagenesis of cloned **Moloney murine leukemia virus** (MuLV) DNA to define a function encoded in the 3' region of the viral **pol** gene and required for efficient integration of viral DNA. One mutant, MuLV-SF1, contained a single base substitution (C to T at base 4950) that resulted in an arginine to cysteine change in a region highly conserved among **retroviruses**. Mutant DNA, introduced into rat cells by cotransfection with a herpes simplex virus thymidine kinase gene (HSV tk), directed production of virus particles with reverse transcriptase activity. Infection of cells with these particles led to synthesis of full-length linear and circular forms of unintegrated viral DNA; however, integrated viral DNA was decreased at least by a factor of 10 when examined by DNA hybridization, and the mutant particles were less efficient then wild-type virus at establishing an infection by a factor of at least 300. **Pseudotypes** formed with the proteins of MuLV-SF1 and the genome of a replication defective **marker** MuLV, carrying the HSV tk gene, were less effective by at least a factor of 100 in producing tk+ colonies than **pseudotypes** formed with proteins encoded by wild-type virus. When the MuLV-SF1 **pseudotypes** did produce tk+ cells, most of the proviruses were integrated aberrantly. We conclude that the MuLV-SF1 **pol** gene is defective for a function that is required for normal integrative recombination and dissociable from DNA synthesis.

=> d his

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E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
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E KUHN RICHARD J/IN
L3 2 S E3
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L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
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L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN

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                E NORTH C L/IN
L20           1 S E3

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     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
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L26          12 S L25 AND (MARKER?/CLM)
L27          10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28         689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29           5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30         283 S L28 AND AY<2000
L31          80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32          16 S L31 AND (MARKER?/CLM)
L33           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38      123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39        1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40          85 S L39 AND (GAG AND POL)
L41           0 S L40 AND (MULTIVALENT)
L42           7 S L40 AND MARKER?

=> s l40 and py<2000
      12131006 PY<2000
L43          67 L40 AND PY<2000

=> d l43,ti,1-67

L43  ANSWER 1 OF 67     MEDLINE on STN
TI   Efficacy and safety analyses of a recombinant human immunodeficiency virus
     type 1 derived vector system.

L43  ANSWER 2 OF 67     MEDLINE on STN
TI   Adeno-retroviral chimeric viruses as in vivo transducing agents.

L43  ANSWER 3 OF 67     MEDLINE on STN
TI   Functional characterization of adenoviral/retroviral chimeric vectors
     and their use for efficient screening of retroviral producer cell lines.

L43  ANSWER 4 OF 67     MEDLINE on STN
TI   Construction of new retroviral producer cells from adenoviral and
     retroviral vectors.

L43  ANSWER 5 OF 67     MEDLINE on STN
TI   A retroviral vector system 'STITCH' in combination with an optimized
     single chain antibody chimeric receptor gene structure allows efficient
     gene transduction and expression in human T lymphocytes.

L43  ANSWER 6 OF 67     MEDLINE on STN
TI   In vitro cell-free conversion of noninfectious Moloney retrovirus
     particles to an infectious form by the addition of the vesicular
     stomatitis virus surrogate envelope G protein.

L43  ANSWER 7 OF 67     MEDLINE on STN
TI   Incorporation of fowl plague virus hemagglutinin into murine leukemia
     virus particles and analysis of the infectivity of the pseudotyped
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retroviruses.

L43 ANSWER 8 OF 67 MEDLINE on STN
TI Noninfectious virus-like particles produced by **Moloney murine leukemia virus**-based **retrovirus** packaging cells deficient in viral envelope become infectious in the presence of lipofection reagents.

L43 ANSWER 9 OF 67 MEDLINE on STN
TI Development of pathological lesions in the central nervous system of transgenic mice expressing the env gene of ts1 **Moloney murine leukemia virus** in the absence of the viral **gag** and **pol** genes and viral replication.

L43 ANSWER 10 OF 67 MEDLINE on STN
TI Placement of tRNA primer on the primer-binding site requires **pol** gene expression in avian but not murine **retroviruses**.

L43 ANSWER 11 OF 67 MEDLINE on STN
TI Transposable elements: how non-LTR retrotransposons do it.

L43 ANSWER 12 OF 67 MEDLINE on STN
TI Defective herpes simplex virus type 1 **vectors** harboring **gag**, **pol**, and env genes can be used to rescue defective **retrovirus vectors**.

L43 ANSWER 13 OF 67 MEDLINE on STN
TI VSV-G-**pseudotyped retroviral** packaging through adenovirus-mediated inducible gene expression.

L43 ANSWER 14 OF 67 MEDLINE on STN
TI Expression of a murine leukemia virus **Gag**-Escherichia coli RNase HI fusion polyprotein significantly inhibits virus spread.

L43 ANSWER 15 OF 67 MEDLINE on STN
TI Production of infectious recombinant **Moloney murine leukemia virus** particles in BHK cells using Semliki Forest virus-derived RNA expression **vectors**.

L43 ANSWER 16 OF 67 MEDLINE on STN
TI A stable human-derived packaging cell line for production of high titer **retrovirus**/vesicular stomatitis virus G **pseudotypes**.

L43 ANSWER 17 OF 67 MEDLINE on STN
TI The zinc finger of nucleocapsid protein of Friend murine leukemia virus is critical for proviral DNA synthesis in vivo.

L43 ANSWER 18 OF 67 MEDLINE on STN
TI Expression of chimeric envelope proteins in helper cell lines and integration into **Moloney murine leukemia virus** particles.

L43 ANSWER 19 OF 67 MEDLINE on STN
TI Inducible, high-level production of infectious murine leukemia **retroviral vector** particles **pseudotyped** with vesicular stomatitis virus G envelope protein.

L43 ANSWER 20 OF 67 MEDLINE on STN
TI A transient three-plasmid expression system for the production of high titer **retroviral vectors**.

L43 ANSWER 21 OF 67 MEDLINE on STN
TI Cytotoxic T lymphocyte and antibody responses generated in rhesus monkeys immunized with **retroviral vector**-transduced fibroblasts expressing human immunodeficiency virus type-1 IIIB ENV/REV proteins.

L43 ANSWER 22 OF 67 MEDLINE on STN
TI **Moloney murine leukemia virus** protease: bacterial expression and characterization of the purified enzyme.

L43 ANSWER 23 OF 67 MEDLINE on STN
TI Vesicular stomatitis virus G glycoprotein **pseudotyped retroviral vectors**: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells.

L43 ANSWER 24 OF 67 MEDLINE on STN
TI Report to the NIH Recombinant DNA Advisory Committee on murine replication-competent **retrovirus** (RCR) assays (February 17, 1993).

L43 ANSWER 25 OF 67 MEDLINE on STN
TI Analysis of a temperature-sensitive mutation affecting the integration protein of **Moloney murine leukemia virus**.

L43 ANSWER 26 OF 67 MEDLINE on STN
TI Regulation of an H-ras-related transcript by parathyroid hormone in rat osteosarcoma cells.

L43 ANSWER 27 OF 67 MEDLINE on STN
TI Bipartite signal for read-through suppression in murine leukemia virus mRNA: an eight-nucleotide purine-rich sequence immediately downstream of the **gag** termination codon followed by an RNA pseudoknot.

L43 ANSWER 28 OF 67 MEDLINE on STN
TI A new **retrovirus** packaging cell for gene transfer constructed from amplified long terminal repeat-free chimeric proviral genes.

L43 ANSWER 29 OF 67 MEDLINE on STN
TI High-frequency intracellular transposition of a defective mammalian provirus detected by an in situ colorimetric assay.

L43 ANSWER 30 OF 67 MEDLINE on STN
TI Gene-transfer into bone marrow cells.

L43 ANSWER 31 OF 67 MEDLINE on STN
TI cis Acting RNA sequences control the **gag-pol** translation readthrough in murine leukemia virus.

L43 ANSWER 32 OF 67 MEDLINE on STN
TI Construction and properties of **retrovirus** packaging cells based on gibbon ape leukemia virus.

L43 ANSWER 33 OF 67 MEDLINE on STN
TI **Retroviral** gene transfer using safe and efficient packaging cell lines.

L43 ANSWER 34 OF 67 MEDLINE on STN
TI Three independent insertions of **retrovirus**-like sequences in the haptoglobin gene cluster of primates.

L43 ANSWER 35 OF 67 MEDLINE on STN
TI A murine cell line producing HTLV-I **pseudotype** virions carrying a selectable marker gene.

L43 ANSWER 36 OF 67 MEDLINE on STN
TI Expression of chimeric tRNA-driven antisense transcripts renders NIH 3T3 cells highly resistant to **Moloney murine leukemia virus** replication.

L43 ANSWER 37 OF 67 MEDLINE on STN
TI Rapid reversion of a deletion mutation in **Moloney murine leukemia virus** by recombination with a closely related endogenous provirus.

L43 ANSWER 38 OF 67 MEDLINE on STN
TI Deletions in a recombinant **retrovirus** genome associated with its expression in embryonal carcinoma cells.

L43 ANSWER 39 OF 67 MEDLINE on STN

TI The effect of specific mutations at and around the **gag-pol** gene junction of Moloney murine leukaemia virus.

L43 ANSWER 40 OF 67 MEDLINE on STN
TI Bovine leukaemia virus packaging cell line for **retrovirus**-mediated gene transfer.

L43 ANSWER 41 OF 67 MEDLINE on STN
TI An insertion mutation in the **pol** gene of **Moloney murine leukemia virus** results in temperature-sensitive **pol** maturation and viral replication.

L43 ANSWER 42 OF 67 MEDLINE on STN
TI Suppression of UAA and UGA termination codons in mutant murine leukemia viruses.

L43 ANSWER 43 OF 67 MEDLINE on STN
TI Translational readthrough of the murine leukemia virus **gag** gene amber codon does not require virus-induced alteration of tRNA.

L43 ANSWER 44 OF 67 MEDLINE on STN
TI Formation of infectious hybrid virions with gibbon ape leukemia virus and human T-cell leukemia virus **retroviral** envelope glycoproteins and the **gag** and **pol** proteins of **Moloney murine leukemia virus**.

L43 ANSWER 45 OF 67 MEDLINE on STN
TI Construction and use of a safe and efficient amphotropic packaging cell line.

L43 ANSWER 46 OF 67 MEDLINE on STN
TI Safe and efficient generation of recombinant **retroviruses** with amphotropic and ecotropic host ranges.

L43 ANSWER 47 OF 67 MEDLINE on STN
TI Selective inhibition of formation of suppressor glutamine tRNA in **Moloney murine leukemia virus**-infected NIH-3T3 cells by Avarol.

L43 ANSWER 48 OF 67 MEDLINE on STN
TI Expression of the **gag-pol** fusion protein of **Moloney murine leukemia virus** without **gag** protein does not induce virion formation or proteolytic processing.

L43 ANSWER 49 OF 67 MEDLINE on STN
TI A safe packaging line for gene transfer: separating viral genes on two different plasmids.

L43 ANSWER 50 OF 67 MEDLINE on STN
TI Inhibition of **retroviral** protease activity by an aspartyl proteinase inhibitor.

L43 ANSWER 51 OF 67 MEDLINE on STN
TI Replication-defective chimeric helper proviruses and factors affecting generation of competent virus: expression of **Moloney murine leukemia virus** structural genes via the metallothionein promoter.

L43 ANSWER 52 OF 67 MEDLINE on STN
TI Oncogenesis by **Moloney murine leukemia virus**.

L43 ANSWER 53 OF 67 MEDLINE on STN
TI Genetic analysis of myeloproliferative leukemia virus, a novel acute leukemogenic replication-defective **retrovirus**.

L43 ANSWER 54 OF 67 MEDLINE on STN
TI Glutamine starvation of murine leukaemia virus-infected cells inhibits the readthrough of the **gag-pol** genes and proteolytic processing of the **gag** polyprotein.

L43 ANSWER 55 OF 67 MEDLINE on STN
TI **Retrovirus**-induced spongiform encephalopathy: the 3'-end long terminal repeat-containing viral sequences influence the incidence of the disease and the specificity of the neurological syndrome.

L43 ANSWER 56 OF 67 MEDLINE on STN
TI The **gag** and **pol** genes of bovine leukemia virus: nucleotide sequence and analysis.

L43 ANSWER 57 OF 67 MEDLINE on STN
TI Point mutations in the P30 domain of the **gag** gene of **Moloney murine leukemia virus**.

L43 ANSWER 58 OF 67 MEDLINE on STN
TI Study of the 78A1 isolate of Moloney murine sarcoma virus. I. Molecular cloning and characterization.

L43 ANSWER 59 OF 67 MEDLINE on STN
TI Generation of a recombinant **Moloney murine leukemia virus** carrying the v-src gene of avian sarcoma virus: transformation in vitro and pathogenesis in vivo.

L43 ANSWER 60 OF 67 MEDLINE on STN
TI Nucleotide sequence of a full-length human endogenous **retroviral** segment.

L43 ANSWER 61 OF 67 MEDLINE on STN
TI A mutant murine leukemia virus with a single missense codon in **pol** is defective in a function affecting integration.

L43 ANSWER 62 OF 67 MEDLINE on STN
TI High-efficiency gene transfer into mammalian cells: generation of helper-free recombinant **retrovirus** with broad mammalian host range.

L43 ANSWER 63 OF 67 MEDLINE on STN
TI Fluidity of a **retrovirus** genome.

L43 ANSWER 64 OF 67 MEDLINE on STN
TI Structure and biological activity of v-raf, a unique oncogene transduced by a **retrovirus**.

L43 ANSWER 65 OF 67 MEDLINE on STN
TI Complete nucleotide sequence and organization of the Moloney murine sarcoma virus genome.

L43 ANSWER 66 OF 67 MEDLINE on STN
TI Characterization of a unique defective type C virus associated with a Moloney leukemia virus-induced splenic T-cell lymphoma cell line.

L43 ANSWER 67 OF 67 MEDLINE on STN
TI Isolation and characterization of a mouse cell line containing a defective **Moloney murine leukemia virus** genome.

=> d l43,cbib,ab,1,18,19,20,33,44,46,49,62

L43 ANSWER 1 OF 67 MEDLINE on STN
1999434719. PubMed ID: 10505094. Efficacy and safety analyses of a recombinant human immunodeficiency virus type 1 derived **vector** system. Chang L J; Urlacher V; Iwakuma T; Cui Y; Zucali J. (Department of Molecular Genetics and Microbiology, University of Florida Brain Institute, Gainesville, USA.) Gene therapy, **(1999 May)** 6 (5) 715-28. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Lentiviruses infect both dividing and nondividing cells. In this study we characterized a lentiviral **vector** system consisting of a packaging

vector (pHP) and a stimulating **vector** (pTV) derived from a recombinant human immunodeficiency virus type 1 (HIV-1). In pHP, the long terminal repeats (LTRs), the 5' untranslated leader and portions of the env and nef genes were deleted. The leader sequence of pHP was substituted with a modified Rous sarcoma virus (RSV) 59 bp leader containing a mutated RSV **gag** AUG and a functional 5' splice site. The pHP construct was found to direct **Gag-Pol** synthesis as efficiently as wild-type HIV-1. The pTV construct contains sequences required for RNA packaging, reverse transcription and integration, but lacks viral genes. Co-transfection of pHP, pTV and a vesicular stomatitis virus G (VSV-G) envelope plasmid produced **vectors** at titers of 10(5)-10(6) transducing units per milliliter in 48 h. Replication-competent virus (RCV) was not detected when deletions were made in the env gene in pHP. The ability of this **vector** system to transduce dividing and nondividing cell in vitro and in vivo was also demonstrated. Compared with a **Moloney murine leukemia virus** (MLV) **vector**, the HP/TV **vectors** transduced human muscle-, kidney-, liver-derived cell lines and CD34+ primary hematopoietic progenitor cells more efficiently. Although the levels of the pTV transgene expression were high soon after transduction, the expression tended to decrease with time due either to the loss of proviral DNA or to the inactivation of promoter activity, which was found to be cell type-dependent. Analyses of extrachromosomal DNA showed that the unintegrated proviral DNA of lentiviral **vectors** survived much longer than that of the **retroviral vectors**. We demonstrate that the HP/TV **vector** is capable of high efficiency transduction and that long-term expression of lentiviral **vectors** is dependent on target cell type, the internal promoter and the transgene itself in the transducing **vector**.

L43 ANSWER 18 OF 67 MEDLINE on STN

96303676. PubMed ID: 8732165. Expression of chimeric envelope proteins in helper cell lines and integration into **Moloney murine leukemia virus** particles. Schnierle B S; Moritz D; Jeschke M; Groner B. (Institute for Experimental Cancer Research, Tumor Biology Center, Freiburg, Germany.) Gene therapy, **(1996 Apr)** 3 (4) 334-42. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB New **retroviral** constructs with a grafted specificity of infection could become useful gene delivery vehicles with applications in systemic gene therapy. We have constructed **retroviral vectors** to target gene transfer to human tumor cells. Chimeric envelope proteins have been expressed to obtain viral particles with a defined specificity of infection. Two tumor cell-specific recognition domains were cloned and fused with the viral envelope gene. A recognition domain specific for ErbB-2 expressing tumor cells was derived from a monoclonal antibody directed against the ErbB-2 receptor in the form of a single chain antibody domain (scFv-erbB-2). The receptor binding domain was derived from the heregulin gene (HRG70). This domain provides recognition specificity for ErbB-3 and ErbB-4 receptor expressing tumor cells. The recognition domains were inserted at the amino terminal end into the **MoMLV** envelope gene. Helper cell lines were established which express the recombinant envelope protein genes, the **gag** and **pol** genes and packageable **retroviral** RNA. The analysis of the helper cell line revealed that the recombinant ErbB-2 scFv-envelope protein was expressed, but not incorporated into viral particles. The scFv-erbB-2 envelope protein was not inserted into the cell membrane and the assembly of **retroviral** particles was not completed. In contrast, the HRG70-envelope protein was expressed on the surface of the helper cells and incorporated into **retroviral** particles. The HRG70-envelope protein, however, did not alter the host range of infection. Only cells expressing the ecotropic viral receptor could be infected.

L43 ANSWER 19 OF 67 MEDLINE on STN

96064119. PubMed ID: 8527479. Inducible, high-level production of infectious murine leukemia **retroviral vector** particles **pseudotyped** with vesicular stomatitis virus G envelope protein. Yang Y; Vanin E F; Whitt M A; Fornerod M; Zwart R; Schneiderman R D; Grosveld G; Nienhuis A

..; (Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.) Human gene therapy, **(1995 Sep)** 6 (9) 1203-13. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Murine leukemia viruses (MuLV) have been adapted for use as gene transfer **vectors** for experimental and human gene therapy applications. Their utility for these purposes has been circumscribed by the limited host range and relatively low titer of available producer clones. **Pseudotyping** of MuLV particles with the vesicular stomatitis virus envelope protein (VSV-G), expressed transiently in cells producing MuLV **Gag** and **Pol** proteins, has yielded **vector** preparations with a broader host range that can be concentrated by ultracentrifugation. We have explored the use of steroid-inducible and tetracycline-modulated promoter systems (necessary because the VSV-G protein is toxic to cells when constitutively expressed) to derive stable producer cell lines capable of substantial production of VSV-G **pseudotyped** MuLV particles. A packaging cell line and producer clones capable of expressing a chimeric transcription factor, composed of the tetracycline repressor (tetR) and the VP16 trans-activating sequences of herpes simplex virus VP16 gene and containing the VSV-G coding sequences linked to a minimal promoter having seven tandem copies of the tetracycline responsive operator (tetO), exhibited high levels of VSV-G protein expression when cultured in the absence of tetracycline. **Vector** particles, produced at titers of 10(5)-10(6) infectious colony forming units per ml (cfu/ml), could be concentrated effectively by ultracentrifugation yielding **vector** preparations having a titer of 10(9) cfu/ml. These cell lines grew normally when VSV-G protein expression was repressed by tetracycline. Such producer clones hold promise for future human gene therapy applications.

L43 ANSWER 20 OF 67 MEDLINE on STN

95206943. PubMed ID: 7899083. A transient three-plasmid expression system for the production of high titer **retroviral vectors**. Soneoka Y; Cannon P M; Ramsdale E E; Griffiths J C; Romano G; Kingsman S M; Kingsman A J. (Department of Biochemistry, University of Oxford, UK.) Nucleic acids research, **(1995 Feb 25)** 23 (4) 628-33. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We have constructed a series of MLV-based **retroviral vectors** and packaging components expressed from the CMV promoter and carried on plasmids containing SV40 origins of replication. These two features greatly enhanced **retroviral** gene expression when introduced into cell lines carrying the SV40 large T antigen. The two packaging components, **gag-pol** and env, were placed on separate plasmids to reduce helper virus formation. Using a highly transfectable human cell line and sodium butyrate to further increase expression of each component, we achieved helper-free viral stocks of approximately 10(7) infectious units/ml by 48 h after transient co-transfection with the three plasmid components. This system can be used both for the generation of high titer **retroviral** stocks for transduction and for the rapid screening of a large number of MLV **gag-pol** or env mutants.

L43 ANSWER 33 OF 67 MEDLINE on STN

91150947. PubMed ID: 2291567. **Retroviral** gene transfer using safe and efficient packaging cell lines. Markowitz D; Hesdorffer C; Ward M; Goff S; Bank A. (Department of Genetics, Columbia University, College of Physicians and Surgeons, New York, New York 10032.) Annals of the New York Academy of Sciences, **(1990)** 612 407-14. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB One of the requirements for the use of **retroviral vectors** in human gene therapy is a packaging cell line which is incapable of producing replication-competent virus and which produces high titers of replication-deficient **vector** virus. Wild-type virus may be produced through recombinational events between the helper virus and a **retroviral vector**. We have constructed an ecotropic packaging cell line, GP + E-86, and an amphotropic packaging cell line, GP + envAm12, in which the viral **gag** and **pol** genes are on one plasmid and the viral env gene is

on another plasmid. Both plasmids contain deletions of the packaging sequence and the 3' LTR. The fragmented helper virus genomes, when introduced into 3T3 cells, produce titers of **retrovirus** which are comparable to the titers produced from packaging cells containing the helper virus genome on a single plasmid. We have found no evidence for the generation of wild-type **retrovirus** using the GP + E-86 and GP + envAm12 packaging lines, either alone or in combination with the N2 **retroviral vector**. We also show that these packaging cell lines can be used to transfer the neoR gene of the N2 **vector** into mouse hematopoietic cells, followed by successful (48-52%), long-term (up to 200 days) transplantation into irradiated recipients. These results indicate that these packaging lines are safe and efficient for use in experiments designed for murine (using GP + E-86) and human (using GP + envAm12) gene therapy.

L43 ANSWER 44 OF 67 MEDLINE on STN

89199801. PubMed ID: 2784836. Formation of infectious hybrid virions with gibbon ape leukemia virus and human T-cell leukemia virus **retroviral** envelope glycoproteins and the **gag** and **pol** proteins of **Moloney murine leukemia virus**. Wilson C; Reitz M S; Okayama H; Eiden M V. (Laboratory of Tumor Cell Biology, National Institute of Cancer, Bethesda, Maryland 20892.) Journal of virology, **(1989 May)** 63 (5) 2374-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The gibbon ape leukemia virus, SEATO strain, and human T-cell leukemia virus type I envelope glycoproteins can be functionally assembled with a **Moloney murine leukemia virus** core into infectious particles. The envelope-host cell receptor interaction is the major determinant of the host cell specificity for these hybrid virions.

L43 ANSWER 46 OF 67 MEDLINE on STN

88320460. PubMed ID: 3413107. Safe and efficient generation of recombinant **retroviruses** with amphotropic and ecotropic host ranges. Danos O; Mulligan R C. (Whitehead Institute for Biomedical Research, Cambridge, MA.) Proceedings of the National Academy of Sciences of the United States of America, **(1988 Sep)** 85 (17) 6460-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have constructed a set of packaging cell lines useful for the generation of helper-free recombinant **retroviruses** with amphotropic and ecotropic host ranges. To eliminate the problems of transfer of packaging functions and helper virus formation encountered with the previously available packaging systems, two mutant **Moloney murine leukemia virus**-derived proviral genomes carrying complementary mutations in the **gag-pol** or env regions were sequentially introduced into NIH 3T3 cells by cotransformation. Both genomes contained a deletion of the psi sequence necessary for the efficient encapsidation of **retroviral** genomes into virus particles and additional alterations at the 3' end of the provirus. We show that the resulting packaging cell lines psi CRIP and psi CRE can be used to isolate clones that stably produce high titers (10(6) colony-forming units/ml) of recombinant **retroviruses** with amphotropic and ecotropic host ranges, respectively. More importantly, we demonstrate that viral producers derived from the packaging cell lines do not transfer the packaging functions, or yield helper virus, even under conditions where existing packaging cell lines can be shown to yield transfer of packaging functions and/or helper virus. These properties of the psi CRIP and psi CRE packaging lines make them particularly valuable reagents for in vivo gene transfer studies aimed at cell lineage analysis and the development of human gene replacement therapies.

L43 ANSWER 49 OF 67 MEDLINE on STN

88155745. PubMed ID: 2831375. A safe packaging line for gene transfer: separating viral genes on two different plasmids. Markowitz D; Goff S; Bank A. (Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, New York 10032.) Journal of virology, **(1988 Apr)** 62 (4) 1120-4. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB A **retrovirus** packaging cell line was constructed by using portions of the **Moloney murine leukemia virus** in which the **gag**, **pol**, and env genes of the helper virus were separated onto two different plasmids and in which the psi packaging signal and 3' long terminal repeat were removed. The plasmid containing the **gag** and **pol** genes and the plasmid containing the env gene were cotransfected into NIH 3T3 cells. Clones that produced high levels of reverse transcriptase and env protein were tested for their ability to package the replication-defective **retrovirus vectors** delta neo and N2. One of the **gag-pol** and env clones (GP+E-86) was able to transfer G418 resistance to recipient cells at a titer of as high as 1.7 X 10(5) when it was used to package delta neo and as high as 4 X 10(6) when it was used to package N2. Supernatants of clones transfected with the intact parent **gag-pol**-env plasmid 3P0 had comparable titers (as high as 6.5 X 10(4) with delta neo; as high as 1.7 X 10(5) with N2). Tests for recombination events that might result in intact **retrovirus** showed no evidence for the generation of replication-competent virus. These results suggest that **gag**, **pol**, and env, when present on different plasmids, may provide an efficient and safe packaging line for use in **retroviral** gene transfer.

L43 ANSWER 62 OF 67 MEDLINE on STN

85038514. PubMed ID: 6093098. High-efficiency gene transfer into mammalian cells: generation of helper-free recombinant **retrovirus** with broad mammalian host range. Cone R D; Mulligan R C. Proceedings of the National Academy of Sciences of the United States of America, **(1984 Oct)** 81 (20) 6349-53. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have constructed a chimeric **retrovirus** genome containing ecotropic **gag-pol** sequences from **Moloney murine leukemia virus** and envelope sequences derived from the amphotropic virus 4070A. This reconstructed genome, termed pMAV-psi-, lacks the psi site required for encapsidation of the viral genome. NIH 3T3 cells transfected with pMAV-psi-, called psi-AM lines, are capable of producing high titer stocks of helper-free recombinant **retrovirus** with amphotropic host range after transfection with recombinant **retroviral vectors** carrying the neomycin phosphotransferase gene. Most transfected psi-AM cells remain helper-free, even after months in culture. psi-AM virus stocks infect nearly all human and murine cell lines tested thus far, as assayed by resistance to the neomycin analogue G418. Southern and RNA blot analyses of psi-AM-infected human cells show that recombinant murine **retroviruses** integrate randomly into genomic DNA as normal proviruses and express high levels of the subgenomic and genomic viral messages in the expected stoichiometry of 1:1.

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(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2

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L9            2 S L8 NOT L6
                E KUHN R J/AU
L10          95 S E3
L11           9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12           8 S L11 NOT (L6 OR L9)
                E JEFFERS S A/AU
L13          33 S E2 OR E5
L14           2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
                E NORTH C L/AU
L15          10 S E3

     FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
                E SANDERS D A/IN
L16           4 S E3
                E FISCHBACH M A/IN
L17           1 S E3
                E KUHN R J/IN
L18           2 S E3
                E JEFFERS S A/IN
L19           3 S E3
                E NORTH C L/IN
L20           1 S E3

     FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

     FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21       13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22        2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23         106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24          28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25          21 S L24 AND AY<2000
L26          12 S L25 AND (MARKER?/CLM)
L27          10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28         689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29           5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30         283 S L28 AND AY<2000
L31          80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32          16 S L31 AND (MARKER?/CLM)
L33           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38      123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39        1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40          85 S L39 AND (GAG AND POL)
L41           0 S L40 AND (MULTIVALENT)
L42           7 S L40 AND MARKER?
L43          67 S L40 AND PY<2000

=> s l38 and (lentivir? expression vector? or lentivir? vector?)
          2819 LENTIVIR?
        610567 EXPRESSION
         99135 VECTOR?
             3 LENTIVIR? EXPRESSION VECTOR?
                 (LENTIVIR?(W)EXPRESSION(W)VECTOR?)
          2819 LENTIVIR?
         99135 VECTOR?
           600 LENTIVIR? VECTOR?
                 (LENTIVIR?(W)VECTOR?)
L44          602 L38 AND (LENTIVIR? EXPRESSION VECTOR? OR LENTIVIR? VECTOR?)

=> s l44 and py<2000
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L45 65 L44 AND PY<2000

=> s l45 and (marker? or selectable or detectable)
255492 MARKER?
2551 SELECTABLE
100234 DETECTABLE
L46 8 L45 AND (MARKER? OR SELECTABLE OR DETECTABLE)

=> d l46,ti,1-8

L46 ANSWER 1 OF 8 MEDLINE on STN
TI Lentiviral gene transfer to the nonhuman primate brain.

L46 ANSWER 2 OF 8 MEDLINE on STN
TI Stable and efficient gene transfer into the mutant retinal pigment epithelial cells of the Mitf(vit) mouse using a **lentiviral vector**.

L46 ANSWER 3 OF 8 MEDLINE on STN
TI Intrabody-mediated knockout of the high-affinity IL-2 receptor in primary human T cells using a bicistronic **lentivirus vector**.

L46 ANSWER 4 OF 8 MEDLINE on STN
TI Identification of a human immunodeficiency virus type 2 (HIV-2) encapsidation determinant and transduction of nondividing human cells by HIV-2-based **lentivirus vectors**.

L46 ANSWER 5 OF 8 MEDLINE on STN
TI Human immunodeficiency virus type 2 **lentivirus vectors** for gene transfer: expression and potential for helper virus-free packaging.

L46 ANSWER 6 OF 8 MEDLINE on STN
TI Defective RNA packaging is responsible for low transduction efficiency of CAEV-based **vectors**.

L46 ANSWER 7 OF 8 MEDLINE on STN
TI Human immunodeficiency virus type 1 **vectors** efficiently transduce human hematopoietic stem cells.

L46 ANSWER 8 OF 8 MEDLINE on STN
TI Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a **lentiviral vector**.

=> d l46,cbib,ab,1-8

L46 ANSWER 1 OF 8 MEDLINE on STN
2000095650. PubMed ID: 10630186. Lentiviral gene transfer to the nonhuman primate brain. Kordower J H; Bloch J; Ma S Y; Chu Y; Palfi S; Roitberg B Z; Emborg M; Hantraye P; Deglon N; Aebischer P. (Department of Neurological Sciences, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612, USA.) Experimental neurology, **(1999 Nov)** 160 (1) 1-16. Journal code: 0370712. ISSN: 0014-4886. Pub. country: United States. Language: English.
AB **Lentiviral vectors** infect quiescent cells and allow for the delivery of genes to discrete brain regions. The present study assessed whether stable lentiviral gene transduction can be achieved in the monkey nigrostriatal system. Three young adult Rhesus monkeys received injections of a **lentiviral vector** encoding for the **marker** gene beta galatosidase (beta Gal). On one side of the brain, each monkey received multiple lentivirus injections into the caudate and putamen. On the opposite side, each animal received a single injection aimed at the substantia nigra. The first two monkeys were sacrificed 1 month postinjection, while the third monkey was sacrificed 3 months postinjection. Robust incorporation of the beta Gal gene was seen in the striatum of all three monkeys. Stereological counts revealed that

positive in monkeys 1 (n = 2) and 3 (n = 1) months later, respectively. Only the third monkey had an injection placed directly into the substantia nigra and 187,308 beta Gal-positive cells were identified in this animal. The injections induced only minor perivascular cuffing and there was no apparent inflammatory response resulting from the lentivirus injections. Double label experiments revealed that between 80 and 87% of the beta Gal-positive cells were neurons. These data indicate that robust transduction of striatal and nigral cells can occur in the nonhuman primate brain for up to 3 months. Studies are now ongoing testing the ability of lentivirus encoding for dopaminergic trophic factors to augment the nigrostriatal system in nonhuman primate models of Parkinson's disease.

L46 ANSWER 2 OF 8 MEDLINE on STN

1999238174. PubMed ID: 10223658. Stable and efficient gene transfer into the mutant retinal pigment epithelial cells of the Mitf(vit) mouse using a **lentiviral vector**. Galileo D S; Hunter K; Smith S B. (Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta 30912-2000, USA.. dgalileo@mail.mcg.edu) . Current eye research, **(1999 Feb)** 18 (2) 135-42. Journal code: 8104312. ISSN: 0271-3683. Pub. country: ENGLAND: United Kingdom. Language: English.

AB PURPOSE: The purpose of the present study was to test whether a **lentiviral vector** encoding the **marker** lacZ gene under the control of the human CMV promoter would stably infect a significant number of RPE cells in the vitiligo mouse. This mouse harbors a mutation in the microphthalmia gene in RPE cells that leads to slow progressive photoreceptor cell degeneration. METHODS: Concentrated **lentiviral vector** HR'CMVlacZ was injected intravitreally into newborn vitiligo mice. Mice were sacrificed at various time points up to two months post-injection and eyes were processed histochemically to detect lacZ expression. RESULTS: The **lentiviral vector** infected predominantly the RPE and resulted in lacZ expression in numerous RPE cells at all times analyzed. CONCLUSIONS: LacZ expression in vitiligo RPE cells appeared to be stable for a period of at least two months. These results raise the possibility of using a similar **lentiviral vector** for introduction of a correct copy of the microphthalmia cDNA into the RPE that may ultimately rescue photoreceptor cells in this mutant mouse.

L46 ANSWER 3 OF 8 MEDLINE on STN

1999014602. PubMed ID: 9797868. Intrabody-mediated knockout of the high-affinity IL-2 receptor in primary human T cells using a bicistronic **lentivirus vector**. Richardson J H; Hofmann W; Sodroski J G; Marasco W A. (Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA.) Gene therapy, **(1998 May)** 5 (5) 635-44. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A bicistronic human immunodeficiency virus type 1 (HIV-1)-based **vector** is described in which the expression of a **selectable marker** and a second gene of interest are forcibly coupled by means of an internal ribosome entry site. The **vector** provides high-level expression of the coselected gene in approximately 90% of transduced cells and has been used to express an endoplasmic reticulum-targeted single-chain antibody (intrabody) directed against a subunit of the interleukin-2 receptor, IL-2R alpha. In the established T cell line Kit225 and also in primary human T cells stably transduced with the intrabody **vector**, the cell surface expression of IL-2R alpha could be reduced to a low or undetectable level. Responsiveness to IL-2 was reduced 10-fold in the IL-2R alpha-negative cells, consistent with a lack of high-affinity IL-2 receptors. **Pseudotyping** of the HIV-1 core with the vesicular stomatitis virus G protein improved particle stability by two- to three-fold and enhanced **vector** entry into established T cell lines up to 230-fold. **Vector** entry into primary human T cells was most efficient when the amphotropic murine leukemia virus envelope was used. The forced, high-expression capability of the bicistronic **vector**, together with the capacity of HIV-1 **vectors** to infect nondividing cells, make this an

deductive tool for the genetic manipulation of primary cell types.

L46 ANSWER 4 OF 8 MEDLINE on STN
1998325168. PubMed ID: 9658096. Identification of a human immunodeficiency virus type 2 (HIV-2) encapsidation determinant and transduction of nondividing human cells by HIV-2-based **lentivirus vectors**. Poeschla E; Gilbert J; Li X; Huang S; Ho A; Wong-Staal F. (Departments of Medicine, University of California at San Diego, La Jolla, California 92093-0665, USA.) Journal of virology, **(1998 Aug)** 72 (8) 6527-36. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Although previous **lentivirus vector** systems have used human immunodeficiency virus type 1 (HIV-1), HIV-2 is less pathogenic in humans and is amenable to pathogenicity testing in a primate model. In this study, an HIV-2 molecular clone that is infectious but apathogenic in macaques was used to first define cis-acting regions that can be deleted to prevent HIV-2 genomic encapsidation and replication without inhibiting viral gene expression. Lentivirus encapsidation determinants are complex and incompletely defined; for HIV-2, some deletions between the major 5' splice donor and the gag open reading frame have been shown to minimally affect encapsidation and replication. We find that a larger deletion (61 to 75 nucleotides) abrogates encapsidation and replication but does not diminish mRNA expression. This deletion was incorporated into a replication-defective, envelope-**pseudotyped**, three-plasmid HIV-2 **lentivirus vector** system that supplies HIV-2 Gag/Pol and accessory proteins in trans from an HIV-2 packaging plasmid. The HIV-2 **vectors** efficiently transduced **marker** genes into human T and monocytoid cell lines and, in contrast to a murine leukemia virus-based **vector**, into growth-arrested HeLa cells and terminally differentiated human macrophages and NTN2 neurons. **Vector** DNA could be detected in HIV-2 **vector**-transduced nondividing CD34(+) CD38(-) human hematopoietic progenitor cells but not in those cells transduced with murine **vectors**. However, stable integration and expression of the reporter gene could not be detected in these hematopoietic progenitors, leaving open the question of the accessibility of these cells to stable lentivirus transduction.

L46 ANSWER 5 OF 8 MEDLINE on STN
1998312641. PubMed ID: 9650621. Human immunodeficiency virus type 2 **lentivirus vectors** for gene transfer: expression and potential for helper virus-free packaging. Arya S K; Zamani M; Kundra P. (Basic Research Laboratory, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA.) Human gene therapy, **(1998 Jun 10)** 9 (9) 1371-80. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.
AB In addition to the long-term expression of the transgene provided by all **retroviral vectors**, lentiviruses present the opportunity to transduce nondividing cells and potentially achieve regulated expression. The development of **lentiviral vectors** requires the design of transfer **vectors** to ferry the transgene with efficient encapsidation of the transgene RNA and with full expression capability, and of a packaging **vector** to provide packaging machinery in trans but without helper virus production. For both **vectors**, a knowledge of packaging signal is required-the signal to be included in the transfer **vector** but excluded from the packaging **vector**. Among the human lentiviruses, human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV-2), we think HIV-2 is better suited for gene transfer than HIV-1. It is less pathogenic and thus safer during design and production; its desirable nuclear import and undesirable cell-cycle arrest functions are segregated on two separate genes. In HIV-1 infection, it is less likely to recombine with the resident HIV-1, and it may itself downregulate HIV-1 expression. Evidently, elements located both upstream and downstream of the splice donor site in the leader sequence participated in RNA encapsidation and these sequences appeared necessary and sufficient. Deletion of both sequence elements resulted in a dramatic curtailment of RNA encapsidation and helper virus production. This was accompanied by some but acceptable loss of gene expression capability. The helper virus-free phenotype and expression capability of the double mutant was maintained upon replacement

of LTR U3 long terminal repeat with a marker cassette containing a transcriptional termination signal and a drug resistance **marker** gene. Deletion of the splice donor site itself had a dramatic negative effect on gene expression, supporting the important role of this element in the life of RNA.

L46 ANSWER 6 OF 8 MEDLINE on STN

1998301988. PubMed ID: 9638141. Defective RNA packaging is responsible for low transduction efficiency of CAEV-based **vectors**. Mselli-Lakhal L; Favier C; Da Silva Teixeira M F; Chettab K; Legras C; Ronfort C; Verdier G; Mornex J F; Cheblounе Y. (Laboratoire Associe de Recherches sur les Lentivirus chez les Petits Ruminants INRA-ENVL, Marcy l'Etoile, France.) Archives of virology, **(1998)** 143 (4) 681-95. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB Replication defective **retroviral vectors** are regularly used for transfer and expression of exogenous genes into dividing cells and in animals. Since lentiviruses are able to infect terminally differentiated and non-dividing cells, their use to produce replication defective **vectors** may overcome this limitation. We developed two replication-defective **lentiviral vectors** based on the genome of Caprine Arthritis Encephalitis Virus (CAEV). The first **vector** (pBNL2) carries the neo and lacZ **marker** genes. Neo gene is expressed from a genomic RNA and lacZ gene from a subgenomic RNA. The second **vector** (pCSHL) carries a single fusion gene encoding both phleomycin resistance and beta-galactosidase activity. Replication-competent CAEV was used as helper virus to provide the viral proteins for transcomplementation of these **vectors**. Our data demonstrated that the genomes of both **vectors** were packaged into CAEV virions and transduced into goat synovial membrane cells following infection. However, the **vector** titers remained 3 to 4 logs lower than those of CAEV. Further analysis showed a lack of accumulation of unspliced pBNL2 RNA into the cytoplasm of producer cells resulting in the packaging of pBNL2 sub-genomic RNA only. In contrast, RNA produced from pCSHL **vector** was correctly transported to the cytoplasm and more efficiently packaged than the pBNL2 sub-genomic RNA as revealed by slot-blot and quantitative RT/PCR analyses. However this higher packaging efficiency of pCSHL genome did not result in a higher transduction efficiency of lacZ gene.

L46 ANSWER 7 OF 8 MEDLINE on STN

1998285735. PubMed ID: 9621037. Human immunodeficiency virus type 1 **vectors** efficiently transduce human hematopoietic stem cells. Sutton R E; Wu H T; Rigg R; Bohnlein E; Brown P O. (Department of Biochemistry and Howard Hughes Medical Institute, Stanford University Medical Center, Stanford, California 94305, USA.. sutton@cmgm.stanford.edu) . Journal of virology, **(1998 Jul)** 72 (7) 5781-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Lentiviruses are potentially advantageous compared to oncoretroviruses as gene transfer agents because they can infect nondividing cells. We demonstrate here that human immunodeficiency virus type 1 (HIV-1)-based **vectors** were highly efficient in transducing purified human hematopoietic stem cells. Transduction rates, measured by **marker** gene expression or by PCR of the integrated provirus, exceeded 50%, and transduction appeared to be independent of mitosis. Derivatives of HIV-1 were constructed to optimize the **vector**, and a deletion of most of Vif and Vpr was required to ensure the long-term persistence of transduced cells with relatively stable expression of the **marker** gene product. These results extend the utility of this **lentivirus vector** system.

L46 ANSWER 8 OF 8 MEDLINE on STN

97030203. PubMed ID: 8876144. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a **lentiviral vector**. Naldini L; Blomer U; Gage F H; Trono D; Verma I M. (Salk Institute for Biological Studies, San Diego, CA 92186-5800, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1996 Oct 15)** 93 (21) 11382-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

We describe the construction of a safe, replication-defective and efficient **lentiviral vector** suitable for in vivo gene delivery. The reverse transcription of the **vector** was found to be a rate-limiting step; therefore, promoting the reaction inside the **vector** particles before delivery significantly enhanced the efficiency of gene transfer. After injection into the brain of adult rats, sustained long-term expression of the transgene was obtained in the absence of **detectable** pathology. A high proportion of the neurons in the areas surrounding the injection sites of the **vector** expressed the transduced beta-galactosidase gene. This pattern was invariant in animals sacrificed several months after a single administration of the **vector**. Transduction occurs by integration of the **vector** genome, as it was abolished by a single amino acid substitution in the catalytic site of the integrase protein incorporated in the **vector**. Development of clinically acceptable derivatives of the **lentiviral vector** may thus enable the sustained delivery of significant amounts of a therapeutic gene product in a wide variety of somatic tissues.

=> d his

(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

FILE 'WPIDS' ENTERED AT 20:03:03 ON 09 MAR 2004
E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

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L21        13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22         2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23          106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24           28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25           21 S L24 AND AY<2000
L26           12 S L25 AND (MARKER?/CLM)
L27           10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28          689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29            5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30          283 S L28 AND AY<2000
L31           80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32           16 S L31 AND (MARKER?/CLM)
L33            0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L34            0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35           10 S LENTIVIRAL EXPRESSION VECTOR?
L36           15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37            3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38       123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39         1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40           85 S L39 AND (GAG AND POL)
L41            0 S L40 AND (MULTIVALENT)
L42            7 S L40 AND MARKER?
L43           67 S L40 AND PY<2000
L44          602 S L38 AND (LENTIVIR? EXPRESSION VECTOR? OR LENTIVIR? VECTOR?)
L45           65 S L44 AND PY<2000
L46            8 S L45 AND (MARKER? OR SELECTABLE OR DETECTABLE)

=> s (ross river virus or ross river alphavirus)
          1895 ROSS
          9668 RIVER
        369440 VIRUS
           269 ROSS RIVER VIRUS
                 (ROSS(W)RIVER(W)VIRUS)
          1895 ROSS
          9668 RIVER
          1098 ALPHAVIRUS
             0 ROSS RIVER ALPHAVIRUS
                 (ROSS(W)RIVER(W)ALPHAVIRUS)
L47         269 (ROSS RIVER VIRUS OR ROSS RIVER ALPHAVIRUS)

=> s l47 and (env?)
        347186 ENV?
L48          39 L47 AND (ENV?)

=> s l48 and (E1 and E2)
'IN' IS NOT A VALID FIELD CODE
             0 "NORTH C J"/IN
             0 "NORTH C J G"/IN
L49          0 L48 AND ("NORTH C J"/IN AND "NORTH C J G"/IN)

=> del l49
DELETE L49? (Y)/N:y

=> s l48 and py<1999
      11672474 PY<1999
L49          23 L48 AND PY<1999

=> d l49,cbib,ab,1-23

L49  ANSWER 1 OF 23     MEDLINE on STN
1999293900.    PubMed ID: 10365566.   Health impact assessments of malaria and
     Ross River virus infection in the Southern Highlands Province of
     Papua New Guinea. Hii J; Dyke T; Dagoro H; Sanders R C. (Papua New Guinea
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Papua and New Guinea medical journal, **(1997 Mar)** 40 (1) 14-25. Journal code: 0376417. ISSN: 0031-1480. Pub. country: Papua New Guinea. Language: English.

AB Malaria at an elevation of 1050 metres is common and highly endemic in the Tagari Valley in the Southern Highlands of Papua New Guinea. Health impact assessments showed that the risks of malaria and epidemic polyarthritis at a gasfield development project in this area were high. Baseline malariometric surveys were conducted in four villages in June and August 1990 and two follow-up surveys (May and December 1991) were made in the village of Nogolitogo near the gasfield pioneer base camp. A total of 941 blood smears were examined. Average malaria prevalence rates decreased with altitude from 56% (at 1050 m) to 9% (at 1700 m) for children 1-9 years of age and from 45% (at 1050 m) to 8% (at 1550 m) for those aged 10 years or more. The spleen rate for children less than 10 years old did not vary significantly with altitude, but average enlarged spleen for all ages decreased with altitude. Mean packed cell volume increased with altitude. Plasmodium falciparum was the most common malaria parasite found and Anopheles punctulatus the predominant vector. Ross River arbovirus (RRV) antibody prevalence was 59%. These results indicate frequent or constant transmission of malaria and pathogenic arboviruses. Entomological and epidemiological data suggested that the vulnerability of the valley community, the receptivity of the **environment** and the health hazards from malaria and RRV were high. Nonimmune Papua New Guineans and expatriate employees face high health hazards; therefore effective preventive measures are required to mitigate epidemics and avoid the likely heightened transmission of malaria and arboviruses caused by the development project.

L49 ANSWER 2 OF 23 MEDLINE on STN

1998403583. PubMed ID: 9734514. A comparison of the diseases caused by **Ross River virus** and Barmah Forest virus. Flexman J P; Smith D W; Mackenzie J S; Fraser J R; Bass S P; Hueston L; Lindsay M D; Cunningham A L. (Royal Perth Hospital, WA.. jameflex@dunamis.rph.uwa.edu.au) . Medical journal of Australia, **(1998 Aug 3)** 169 (3) 159-63. Ref: 48. Journal code: 0400714. ISSN: 0025-729X. Pub. country: Australia. Language: English.

AB Barmah Forest virus (BFV) and **Ross River virus** (RRV) are mosquito-borne viruses with similar vectors and **environmental** requirements. They cause diseases characterised by arthralgia, arthritis and myalgia, often accompanied by fever and rash. Arthritis is more common and more prominent in RRV disease and rash is more common and florid with BFV infection, although the diseases cannot be reliably distinguished by their clinical symptoms. Diagnosis is based on serological tests and a definite diagnosis of recent infection requires the demonstration of rising titres of IgG. Arthralgia, myalgia and lethargy may continue for at least six months in up to half of patients with RRV, but in only about 10% of patients with BFV. Both diseases are managed symptomatically.

L49 ANSWER 3 OF 23 MEDLINE on STN

1998105802. PubMed ID: 9445057. Structural localization of the E3 glycoprotein in attenuated Sindbis virus mutants. Paredes A M; Heidner H; Thuman-Commike P; Prasad B V; Johnston R E; Chiu W. (National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.. angel@tiger.3dem.bioch.bcm.tmc.edu) . Journal of virology, **(1998 Feb)** 72 (2) 1534-41. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have determined the three-dimensional structures of the wild-type Sindbis virus and two of its mutants that retain the E3 sequence within PE2. Using difference imaging between these mutants and the wild-type virus, we have assigned a location for the 64-amino-acid sequence corresponding to E3 in the mutant spike complex. In the wild-type virus, the spike is composed of an E1-E2 heterotrimer. The E3 protein was found to protrude midway between the center of the spike complex and the tips.

Based on these results and the work of others, we propose a distribution for the functional domains of the spike proteins within the structure of wild-type Sindbis virus. Within the structure of the virus, the E1 domains form the central portion of the spike complex, while the tips are formed by the E2 domains that flare out from the center of the complex. The structural similarity between these Sindbis virus mutants and **Ross River virus** suggests that E3 may also be present in the latter, which is also a member of the Alphavirus genus.

L49 ANSWER 4 OF 23 MEDLINE on STN

1998105788. PubMed ID: 9445043. Molecular genetic study of the interaction of Sindbis virus E2 with **Ross River virus** E1 for virus budding. Yao J; Strauss E G; Strauss J H. (Division of Biology, California Institute of Technology, Pasadena 91125, USA.) Journal of virology, **(1998 Feb)** 72 (2) 1418-23. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Glycoprotein PE2 of Sindbis virus will form a heterodimer with glycoprotein E1 of **Ross River virus** that is cleaved to an E2/E1 heterodimer and transported to the cell plasma membrane, but this chimeric heterodimer fails to interact with Sindbis virus nucleocapsids, and very little budding to produce mature virus occurs upon infection with chimeric viruses. We have isolated in both Sindbis virus E2 and in **Ross River virus** E1 a series of suppressing mutations that adapt these two proteins to one another and allow increased levels of chimeric virus production. Two adaptive E1 changes in an ectodomain immediately adjacent to the membrane anchor and five adaptive E2 changes in a 12-residue ectodomain centered on Asp-242 have been identified. One change in **Ross River virus** E1 (Gln-411-->Leu) and one change in Sindbis virus E2 (Asp-248-->Tyr) were investigated in detail. Each change individually leads to about a 10-fold increase in virus production, and combined the two changes lead to a 100-fold increase in virus. During passage of a chimeric virus containing **Ross River virus** E1 and Sindbis virus E2, the E2 change was first selected, followed by the E1 change. Heterodimers containing these two adaptive mutations have a demonstrably increased degree of interaction with Sindbis virus nucleocapsids. In the parental chimera, no interaction between heterodimers and capsids was visible at the plasma membrane in electron microscopic studies, whereas alignment of nucleocapsids along the plasma membrane, indicating interaction of heterodimers with nucleocapsids, was readily seen in the adapted chimera. The significance of these findings in light of our current understanding of alphavirus budding is discussed.

L49 ANSWER 5 OF 23 MEDLINE on STN

97433152. PubMed ID: 9288821. Genetic stability among temporally and geographically diverse isolates of Barmah Forest virus. Poidinger M; Roy S; Hall R A; Turley P J; Scherret J H; Lindsay M D; Broom A K; Mackenzie J S. (Department of Microbiology, University of Queensland, Brisbane, Australia.) American journal of tropical medicine and hygiene, **(1997 Aug)** 57 (2) 230-4. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB An increase in the incidence of polyarthritis caused by Barmah Forest (BF) virus, and its recent emergence into Western Australia, prompted a study of the molecular epidemiology of this Australian mosquito-borne alphavirus. The nucleotide sequence of a 500-basepair region of the 3' end of the **envelope** (E2) gene of the prototype BF virus strain (BH2193) was compared with other members of the alphavirus genus, and to a panel of isolates of BF virus collected more for than 20 years from different geographic regions of Australia. The BF virus was shown to be genetically distinct from other members of the alphavirus genus. A high degree of sequence homology (98-100%) was found between the BF isolates, with no evidence of geographic or temporal divergence. This nucleotide homogeneity was similar to that observed with other Australian mosquito-borne viruses with avian vertebrate hosts, such as Sindbis, Murray Valley, and Kunjin viruses, but it contrasts to the heterogeneity reported for **Ross River virus**, an alphavirus with mammalian vertebrate hosts.

L49 ANSWER 6 OF 23 MEDLINE on STN
97213948. PubMed ID: 9060637. Sindbis virus replicons and Sindbis virus: assembly of chimeras and of particles deficient in virus RNA. Frolov I; Frolova E; Schlesinger S. (Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110-1093, USA.) Journal of virology, **(1997 Apr)** 71 (4) 2819-29. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Alphaviruses are a well-characterized group of positive-strand RNA viruses. The identification of cis-acting elements in their genomes and their replication strategy have made them useful as vectors for the expression of heterologous genes. In infected cells, the nonstructural proteins, required for replication and transcription of the viral genes, are translated from the genomic RNA; the structural proteins, the capsid protein that interacts with the RNA to form the nucleocapsid and the proteins embedded in the lipid **envelope**, are translated from a subgenomic mRNA and can be replaced by heterologous genes. Such modified genomes are self-replicating (replicons); they can be introduced into the cells by transfection and can also be packaged into extracellular particles with defective helper (DH) RNAs. The particular DH RNA determines how well it is replicated and to what extent it is packaged. One potential complication of this system has been that recombination between the replicon genome and the DH RNA may occur. The studies described here were designed to prevent recombination by expressing the capsid protein from one DH RNA and the virus membrane proteins from a second helper RNA. Recombination to yield a nonsegmented infectious virus genome would then require several independent crossover events. There is a translational enhancer located downstream of the initiating AUG in the RNA of the capsid gene that had to be conserved in the second helper to achieve high-level expression of the viral glycoproteins. For this reason, we modified the capsid protein gene in two ways: the first was to use the capsid protein gene from a different alphavirus, **Ross River virus**, and the second was to make deletions in that gene to maintain the translational enhancer in the RNA but to eliminate the positively charged region in the protein that should be essential for the specific and nonspecific interactions with RNA. Transfections with replicon RNA and the deleted chimeric DH RNA as the only helper resulted in the high-level production of particles that were almost completely devoid of RNA. The inclusion of a helper expressing an intact Sindbis virus capsid protein gene led to the production of high levels of packaged replicons. Recombinants were not detected even after several undiluted passages.

L49 ANSWER 7 OF 23 MEDLINE on STN
97064087. PubMed ID: 8903211. An outbreak of **Ross River virus** disease in Southwestern Australia. Lindsay M; Oliveira N; Jasinska E; Johansen C; Harrington S; Wright A E; Smith D. (University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands, Western Australia.) Emerging infectious diseases, **(1996 Apr-Jun)** 2 (2) 117-20. Journal code: 9508155. ISSN: 1080-6040. Pub. country: United States. Language: English.

L49 ANSWER 8 OF 23 MEDLINE on STN
97048073. PubMed ID: 8892914. Interactions between PE2, E1, and 6K required for assembly of alphaviruses studied with chimeric viruses. Yao J S; Strauss E G; Strauss J H. (Division of Biology, California Institute of Technology, Pasadena 91125, USA.) Journal of virology, **(1996 Nov)** 70 (11) 7910-20. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB During the assembly of alphaviruses, a preassembled nucleocapsid buds through the cell plasma membrane to acquire an **envelope** containing two virally encoded glycoproteins, E2 and E1. Using two chimeric viruses, we have studied interactions between E1, E2, and a viral peptide called 6K, which are required for budding. A chimeric Sindbis virus (SIN) in which the 6K gene had been replaced with that from **Ross River virus** (RR) produced wild-type levels of nucleocapsids and abundant PE2/E1 heterodimers that were processed and transported to the cell surface.

However, only about 1% as much chimeric virus as wild type virus was assembled, demonstrating that there is a sequence-specific interaction between 6K and the glycoproteins required for efficient virus assembly. In addition, the conformation of E1 in the E2/E1 heterodimer on the cell surface was different for the chimeric virus from that for the wild type, suggesting that one function of 6K is to promote proper folding of E1 in the heterodimer. A second chimeric SIN, in which both the 6K and E1 genes, as well as the 3' nontranslated region, were replaced with the corresponding regions of RR also resulted in the production of large numbers of intracellular nucleocapsids and of PE2/E1 heterodimers that were cleaved and transported to the cell surface. Budding of this chimera was severely impaired, however, and the yield of the chimera was only approximately 10(-7) of the SIN yield in a parallel infection. The conformation of the SIN E2/RR E1 heterodimer on the cell surface was different from that of the SIN E2/SIN E1 heterodimer, and no interaction between viral glycoproteins and nucleocapsids at the cell plasma membrane could be detected in the electron microscope. We suggest that proper folding of the E2/E1 heterodimer must occur before the E2 tail is positioned properly in the cytoplasm for budding and before heterodimer trimerization can occur to drive virus budding.

L49 ANSWER 9 OF 23 MEDLINE on STN

97041949. PubMed ID: 8887220. Evaluation of Mesocyclops aspericornis (Cyclopoida:Cyclopidae) and Toxorhynchites speciosus as integrated predators of mosquitoes in tire habitats in Queensland. Brown M D; Hendrikz J K; Greenwood J G; Kay B H. (Queensland Institute of Medical Research, Royal Brisbane Hospital, Australia.) Journal of the American Mosquito Control Association, **(1996 Sep)** 12 (3 Pt 1) 414-20. Journal code: 8511299. ISSN: 8756-971X. Pub. country: United States. Language: English.

AB This study addressed biological control of peridomestic Aedes notoscriptus, known to be a highly effective colonizer of tire habitats and a possible vector of **Ross River virus**. A laboratory trial of the compatibility of the predators Mesocyclops aspericornis and Toxorhynchites speciosus in small container habitats showed that 4th-instar Tx. speciosus did not significantly affect M. aspericornis mortality. Introduced M. aspericornis and naturally occurring Tx. speciosus were found to form a compatible predator pair for reduction of larval Ae. notoscriptus and Culex quinquefasciatus populations in tire habitats. Over 22 months of field survey, 97% of tires without predators contained mosquito larvae, at a median density of 43 larvae/liter. By comparison, 51% of tires containing both predator species held mosquito larvae at a median density of 4 larvae/liter. Predation by Tx. speciosus persisted for the duration of the study. The inability of the Lake Kurwongbah strain of M. aspericornis to tolerate temperatures of < or = 10 degrees C, which are prevalent in Brisbane during winter, resulted in a failure to deliver persistent reduction of mosquitoes in tires. The temperature-dependent population characteristics of M. aspericornis emphasize the long-recognized importance of matching a biological control candidate's physiological requirements to the **environment** in which control is sought.

L49 ANSWER 10 OF 23 MEDLINE on STN

96190574. PubMed ID: 8627696. Entry kinetics and mouse virulence of **Ross River virus** mutants altered in neutralization epitopes. Vrati S; Kerr P J; Weir R C; Dalgarno L. (Division of Biochemistry and Molecular Biology, Faculty of Science, Australian National University, Canberra, Australia.) Journal of virology, **(1996 Mar)** 70 (3) 1745-50. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Previously we identified the locations of three neutralization epitopes (a, b1 and b2) of **Ross River virus** (RRV) by sequencing a number of variants resistant to monoclonal antibody neutralization which were found to have single amino acid substitutions in the E2 protein (S. Vrati, C.A. Fernon, L. Dalgarno, and R.C. Weir, Virology 162:346-353, 1988). We have now studied the biological properties of these variants in BHK cells

and small virulence in mice. While variants altered in epitopes a and/or b1 showed no difference, variants altered in epitope b2, including a triple variant altered in epitopes a, b1, and b2, showed rapid penetration but retarded kinetics of growth and RNA and protein synthesis in BHK cells compared with RRV T48, the parent virus. Variants altered in epitopes a and/or b1 showed no change in mouse virulence. However, two of the six epitope b2 variants examined had attenuated mouse virulence. They had a four- to fivefold-higher 50% lethal dose (LD50), although no change in the average survival time of infected mice was observed. These variants grew to titers in mouse tissues similar to those of RRV T48. The ID50 of the triple variant was unchanged, but infected mice had an increased average survival time. This variant produced lower levels of viremia in infected mice. On the basis of these findings we propose that both the receptor binding site and neutralization epitopes of RRV are nearby or in the same domain of the E2 protein.

L49 ANSWER 11 OF 23 MEDLINE on STN
96068673. PubMed ID: 7479858. Putative receptor binding sites on alphaviruses as visualized by cryoelectron microscopy. Smith T J; Cheng R H; Olson N H; Peterson P; Chase E; Kuhn R J; Baker T S. (Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392, USA.) Proceedings of the National Academy of Sciences of the United States of America, **(1995 Nov 7)** 92 (23) 10648-52. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB The structures of Sindbis virus and **Ross River virus** complexed with Fab fragments from monoclonal antibodies have been determined from cryoelectron micrographs. Both antibodies chosen for this study bind to regions of the virions that have been implicated in cell-receptor recognition and recognize epitopes on the E2 glycoprotein. The two structures show that the Fab fragments bind to the outermost tip of the trimeric **envelope** spike protein. Hence, the same region of both the Sindbis virus and **Ross River virus envelope** spike is composed of E2 and is involved in recognition of the cellular receptor.

L49 ANSWER 12 OF 23 MEDLINE on STN
95171457. PubMed ID: 7867069. Nucleocapsid and glycoprotein organization in an **enveloped** virus. Cheng R H; Kuhn R J; Olson N H; Rossmann M G; Choi H K; Smith T J; Baker T S. (Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907.) Cell, **(1995 Feb 24)** 80 (4) 621-30. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.
AB Alphaviruses are a group of icosahedral, positive-strand RNA, **enveloped** viruses. The membrane bilayer, which surrounds the approximately 400 A diameter nucleocapsid, is penetrated by 80 spikes arranged in a T = 4 lattice. Each spike is a trimer of heterodimers consisting of glycoproteins E1 and E2. Cryoelectron microscopy and image reconstruction of **Ross River virus** showed that the T = 4 quaternary structure of the nucleocapsid consists of pentamer and hexamer clusters of the capsid protein, but not dimers, as have been observed in several crystallographic studies. The E1-E2 heterodimers form one-to-one associations with the nucleocapsid monomers across the lipid bilayer. Knowledge of the atomic structure of the capsid protein and our reconstruction allows us to identify capsid-protein residues that interact with the RNA, the glycoproteins, and adjacent capsid-proteins.

L49 ANSWER 13 OF 23 MEDLINE on STN
95060425. PubMed ID: 7970354. Do Ross River and dengue viruses pose a threat to New Zealand?. Maguire T. (Health Research Council of New Zealand's Virus Research Unit, University of Otago, Dunedin.) New Zealand medical journal, **(1994 Nov 9)** 107 (989) 448-50. Journal code: 0401067. ISSN: 0028-8446. Pub. country: New Zealand. Language: English.
AB AIM. To determine the prevalence of antibodies to Ross River and dengue viruses in sera from New Zealand residents and travellers and to assess the potential of local mosquitoes to act as vectors of these viruses. METHOD. Serum specimens from several population groups were examined by haemagglutination-inhibition and neutralisation tests for antibodies to

Ross River and dengue viruses between 1979 and 1990. During this period dengue was active in South East Asia, Australia and the Pacific, and a major epidemic of Ross River infection occurred in the Pacific. Two New Zealand mosquito species were tested for their ability to transmit by bite after they had been fed or injected with these viruses. RESULTS. Ten percent of 1869 sera from patients suspected of contracting dengue, and 43% of 183 patients suspected of contracting **Ross River virus**, while overseas, were antibody positive. Many patients showed antibody rises which indicated that they were probably viraemic on entry to this country. Dengue viruses were isolated in Dunedin from two patients with dengue haemorrhagic fever contracted overseas. Antibody studies of persons who had not travelled outside New Zealand provided no evidence of local transmission of these viruses. Two local mosquitoes, Aedes notoscriptus from the Auckland area, and Aedes australis from the Otago area, were able to transmit one or both these viruses under laboratory conditions. CONCLUSIONS. The serological studies showed that both Ross River and dengue viruses have probably been introduced into New Zealand by viraemic travellers on many occasions. Although some local mosquitoes can transmit these viruses in the laboratory, there is no evidence of local spread of virus from these imported cases. Changing **environmental** conditions such as global warming with concomitant effects on vector distribution, increasingly rapid air travel by viraemic persons and the accidental introduction of new vector mosquitoes, particularly Aedes albopictus, could pose a threat in view of the high percentage of New Zealand residents with no protective antibody.

L49 ANSWER 14 OF 23 MEDLINE on STN

94149818. PubMed ID: 7508993. Nucleocapsid-glycoprotein interactions required for assembly of alphaviruses. Lopez S; Yao J S; Kuhn R J; Strauss E G; Strauss J H. (Division of Biology, California Institute of Technology, Pasadena 91125.) Journal of virology, **(1994 Mar)** 68 (3) 1316-23. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB We have studied interactions between nucleocapsids and glycoproteins required for budding of alphaviruses, using **Ross River virus**-Sindbis virus chimeras in which the nucleocapsid protein is derived from one virus and the **envelope** glycoproteins are derived from the second virus. A virus containing the **Ross River virus** genome in which the capsid protein had been replaced with that from Sindbis virus was almost nonviable. Nucleocapsids formed in normal numbers in the infected cell, but very little virus was released from the cell. There are 11 amino acid differences between **Ross River virus** and Sindbis virus in their 33-residue E2 cytoplasmic domains. Site-specific mutagenesis was used to change 9 of these 11 amino acids in the chimera from the **Ross River virus** to the Sindbis virus sequence in an attempt to adapt the E2 of the chimera to the nucleocapsid. The resulting mutant chimera grew 4 orders of magnitude better than the parental chimeric virus. This finding provides direct evidence for a sequence-specific interaction between the nucleocapsid and the E2 cytoplasmic domain during virus budding. The mutated chimeric virus readily gave rise to large-plaque variants that grew almost as well as **Ross River virus**, suggesting that additional single amino acid substitutions in the structural proteins can further enhance the interactions between the disparate capsid and the glycoproteins. Unexpectedly, change of E2 residue 394 from lysine (**Ross River virus**) to glutamic acid (Sindbis virus) was deleterious for the chimera, suggesting that in addition to its role in nucleocapsid-E2 interactions, the N-terminal part of the E2 cytoplasmic domain may be involved in glycoprotein-glycoprotein interactions required to assemble the glycoprotein spikes. The reciprocal chimera, Sindbis virus containing the **Ross River virus** capsid, also grew poorly. Suppressor mutations arose readily in this chimera, producing a virus that grew moderately well and that formed larger plaques.

L49 ANSWER 15 OF 23 MEDLINE on STN

94106709. PubMed ID: 8279636. **Ross River virus** isolations from mosquitoes in arid regions of Western Australia: implication of vertical

transmission as a means of persistence of the virus. Lindsey H S; Bloom M K; Wright A E; Johansen C A; Mackenzie J S. (Department of Microbiology, University of Western Australia, Queen Elizabeth II Medical Centre, Nedlands.) American journal of tropical medicine and hygiene, **(1993 Dec)** 49 (6) 686-96. Journal code: 0370507. ISSN: 0002-9637. Pub. country: United States. Language: English.

AB Outbreaks of mosquito-borne Ross River (RR) virus disease (epidemic polyarthritis) occur suddenly in the arid north and interior of the State of Western Australia, often within a few weeks of heavy rainfall. Between outbreaks, these regions may undergo long periods of drought, with little or no mosquito or arbovirus activity. The means by which RR virus is reintroduced or reactivated in these areas when **environmental** conditions favor mosquito-borne virus activity are unknown. In this paper, we describe isolations of RR virus from eight mosquito species trapped at two different locations, one coastal and one inland, in the arid Pilbara region of Western Australia, prior to outbreaks of epidemic polyarthritis. The isolation of RR virus has not been previously reported for five of these species and the isolations from the other three species are new records for Western Australia. The timing and number of isolations of RR virus from Aedes (Ochlerotatus) vigilax (Skuse, 1889) implicate that species as a vector of the virus on the Pilbara coast. Significantly, RR virus was isolated from pools of male Ae. vigilax and male Ae. (Macleaya) tremulus (Theobald, 1903) mosquitoes. This is the first report of RR virus (or other Australian arbovirus) isolates from wild-caught male mosquitoes. Both Ae. vigilax and Ae. tremulus have desiccation-resistant eggs that can survive long periods of drought, making them ideal candidates for the overwintering of arboviruses. The findings implicate vertical transmission as a means of persistence of RR virus in arid regions of Australia and therefore offer a likely explanation for the sudden recurrence of virus activity after heavy rains.

L49 ANSWER 16 OF 23 MEDLINE on STN

93174957. PubMed ID: 7679860. **Ross River virus** variants selected during passage in chick embryo fibroblasts: serological, genetic, and biological changes. Kerr P J; Weir R C; Dalgarno L. (Department of Biochemistry, Faculty of Science, Australian National University, Canberra.) Virology, **(1993 Mar)** 193 (1) 446-9. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB Serial passage of **Ross River virus** in chick embryo fibroblasts selected for virus variants altered in their reactions to neutralizing monoclonal antibodies. The E1 and E2 genes of each antigenic variant were sequenced; single nucleotide changes were found in E2 leading to amino acid substitutions at either residue 4 (Glu-->Lys) or 218 (Asn-->Lys); no changes were found in the E1 gene. Variants with the substitution at E2 residue 218 replicated less efficiently in 1-day-old mice than did the parental strain. The variant changed at E2 residue 4 showed little alteration in replication efficiency in mice. Similar genotypic or phenotypic changes were not found in virus passaged serially in human or mosquito cell lines.

L49 ANSWER 17 OF 23 MEDLINE on STN

92122277. PubMed ID: 1685192. Mosquito (Diptera: Culicidae) and arbovirus activity on the south coast of New South Wales, Australia, in 1985-1988. Russell R C; Cloonan M J; Wells P J; Vale T G. (Medical Entomology Unit, University of Sydney, Westmead Hospital, NSW, Australia.) Journal of medical entomology, **(1991 Nov)** 28 (6) 796-804. Journal code: 0375400. ISSN: 0022-2585. Pub. country: United States. Language: English.

AB An investigation of the seasonal activity of adult mosquitoes and arboviruses in two native forests revealed a mosquito fauna comprising 33 species for the Mogo State Forest-Batemans Bay locality and 34 species for the Termeil State Forest. The mosquito Aedes vigilax was the most abundant species in salt marshes at Mogo State Forest-Batemans Bay, whereas the freshwater species Anopheles annulipes s.l. and Coquillettidia linealis were most abundant at Termeil State Forest. Major faunal differences between the two **environments** reflected the extensive saline marsh and mudflat estuarine habitats at Batemans Bay and the predominantly

freshwater swamp, marsh, and lake habitats of Termeil State Forest. A total of 218 virus isolates was recovered from the mosquitoes, comprising **Ross River virus** (86 isolates), Gan Gan virus (123 isolates), Termeil virus (2 isolates), and 7 unidentified viruses. The period of greatest arbovirus activity was from midsummer through midautumn and coincided with the overall peak activity of the most common mosquito species. Ae. vigilax yielded most isolates of Ross River and Gan Gan viruses and appeared to be the vector of greatest public health concern.

L49 ANSWER 18 OF 23 MEDLINE on STN

88179556. PubMed ID: 2833022. Genome sequences of a mouse-avirulent and a mouse-virulent strain of **Ross River virus**. Faragher S G; Meek A D; Rice C M; Dalgarno L. (Biochemistry Department, Faculty of Science, Australian National University, Canberra.) Virology, **(1988 Apr)** 163 (2) 509-26. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The nucleotide sequence of the genomic RNA of a mouse-avirulent strain of **Ross River virus**, RRV NB5092 (isolated in 1969), has been determined and the corresponding sequence for the prototype mouse-virulent strain, RRV T48 (isolated in 1959), has been completed. The RRV NB5092 genome is approximately 11,674 nucleotides in length, compared with 11,853 nucleotides for RRV T48. RRV NB5092 and RRV T48 have the same genome organization. For both viruses an untranslated region of 80 nucleotides at the 5' end of the genome is followed by a 7440-nucleotide open reading frame which is interrupted after 5586 nucleotides by a single opal termination codon. By homology with other alphaviruses, the 5586-nucleotide open reading frame encodes the nonstructural proteins nsP1, nsP2, and nsP3; a fourth nonstructural protein, nsP4, is produced by read-through of the opal codon. The RRV nonstructural proteins show strong homology with the corresponding proteins of Sindbis virus and Semliki Forest virus in terms of size, net charge, and hydropathy characteristics. However, homology is not uniform between or within the proteins; nsP1, nsP2, and nsP4 contain extended domains which are highly conserved between alphaviruses, while the C-terminal region of nsP3 shows little conservation in sequence or length between alphaviruses. An untranslated "junction" region of 44 nucleotides (for RRV NB5092) or 47 nucleotides (for RRV T48) separates the nonstructural and structural protein coding regions. The structural proteins (capsid-E3-E2-6K-E1) are translated from an open reading frame of 3762 nucleotides which is followed by a 3'-untranslated region of approximately 348 nucleotides (for RRV NB5092) or 524 nucleotides (for RRV T48). Excluding deletions and insertions, the genomes of RRV NB5092 and RRV T48 differ at 284 nucleotides, representing a sequence divergence of 2.38%. Sequence deletions or insertions were found only in the noncoding regions and include a 173-nucleotide deletion in the 3'-untranslated region of RRV NB5092, compared with RRV T48. In the coding regions, most of the nucleotide differences are silent; there are 36 amino acid differences in the nonstructural proteins and 12 in the structural proteins. The distribution of amino acid differences between the two RRV strains correlates with the location of domains which are poorly conserved in sequence between alphaviruses. The possible role of amino acid differences in **envelope** glycoproteins E1 and E2 in determining the different antigenic and biological properties of RRV NB5092 and RRV T48 is discussed.

L49 ANSWER 19 OF 23 MEDLINE on STN

86209986. PubMed ID: 3010551. **Ross River virus** mutant with a deletion in the E2 gene: properties of the virion, virus-specific macromolecule synthesis, and attenuation of virulence for mice. Vrati S; Faragher S G; Weir R C; Dalgarno L. Virology, **(1986 Jun)** 151 (2) 222-32. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB A mutant of RRV T48 the prototype strain of **Ross River virus** has been isolated with a 21-nucleotide deletion in the gene coding for the **envelope** glycoprotein E2. Direct sequencing of the 26 S subgenomic RNA, together with HaeIII and TaqI restriction digest analysis of cDNA to RNAs

consistent with the deletion being the only major alteration in the mutant genome. The E2 protein of RRV dE2 virions had a higher electrophoretic mobility than that of RRV T48 E2 protein. Neither RRV dE2 nor RRV T48 virions contained more than trace amounts of E3, the small **envelope** glycoprotein found in Semliki Forest virus. RRV dE2 generated small plaques on Vero cell monolayers; plaque formation was not temperature-sensitive between 32 and 41 degrees. By comparison with RRV T48 the infectivity of RRV dE2 virions was thermolabile at 50 degrees. In BHK cells RRV dE2 grew with similar kinetics to RRV T48. Rates of synthesis of 26 S RNA and 49 S RNA were higher in cells infected with RRV dE2 than in cells infected with RRV T48. Virus-specific protein synthesis and shut-down of host protein synthesis occurred 2-3 hr earlier in RRV dE2-infected cells than in cells infected with RRV T48. Minor differences between the two viruses were observed in the profiles of virus-specific proteins generated in infected cells. In day-old mice RRV dE2 induced less severe symptoms of hind leg paralysis than did RRV T48. A small increase in LD50 and average survival time was observed in RRV dE2-infected mice by comparison with RRV T48 infected mice. Peak titers reached by RRV dE2 in the hind leg muscle, brain, and blood of day-old mice were 3-4 log units less than the titers reached during infection with RRV T48. In week-old mice the differences in virulence between the two strains were magnified: RRV dE2 induced no detectable symptoms even when injected at high doses (8 X 10(6) PFU) whereas the LD50 and average survival time for RRV T48 were unchanged from those in day-old mice. Peak RRV dE2 titers in hind leg muscle, brain, and blood, respectively, were 2, 5, and 5 log units less than the corresponding titers for RRV T48. Peak muscle titers reached by RRV dE2 were similar (approximately 10(8) PFU/g tissue) in day-old mice where lethality was high and in week-old mice where the virus was avirulent.(ABSTRACT TRUNCATED AT 400 WORDS)

L49 ANSWER 20 OF 23 MEDLINE on STN

83303839. PubMed ID: 6310876. **Ross River virus** 26 s RNA: complete nucleotide sequence and deduced sequence of the encoded structural proteins. Dalgarno L; Rice C M; Strauss J H. Virology, **(1983 Aug)** 129 (1) 170-87. Journal code: 0110674. ISSN: 0042-6822. Pub. country: United States. Language: English.

AB The complete sequence of the 26 S RNA of **Ross River virus** (T48 strain) has been obtained and from this the amino acid sequences of the encoded structural proteins have been deduced. These include a basic capsid protein and two **envelope** glycoproteins. The nucleotide sequence was obtained by chemical sequence analysis of both single-stranded and double-stranded cDNA made to RNA and the sequence data so obtained was rapidly aligned by making use of the protein homology found among the alphaviruses. The polyprotein precursor encoded by the 26 S RNA of **Ross River virus** is 75% homologous to that of Semliki Forest virus and 48% homologous to that of Sindbis virus. The extent of homology is not uniform within a protein or between proteins and this is discussed with respect to the possible function of the various polypeptide domains in the virus life cycle. In each case the putative attachment site of the amino proximal carbohydrate chains of the three glycoproteins is conserved, whereas the attachment site of a second chain, if present, is not conserved. The 3'-untranslated region of **Ross River virus** RNA is 524 nucleotides long. It contains a sequence of about 50 nucleotides in length which is present in four copies but which is not shared with other alphaviruses examined.

L49 ANSWER 21 OF 23 MEDLINE on STN

81009688. PubMed ID: 7411679. The urban mosquitoes of Suva, Fiji: seasonal incidence and evaluation of **environmental** sanitation and ULV spraying for their control. Goettel M S; Toohey M K; Pillai J S. Journal of tropical medicine and hygiene, **(1980 Aug)** 83 (4) 165-71. Journal code: 0406044. ISSN: 0022-5304. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Larval surveys and oviposition traps were used to monitor urban mosquito populations in two adjacent transects in Suva, Fiji between May 1978 and

August 1979. Populations of Aedes aegypti and Ae. pseudoscutellaris fluctuated seasonally with changes in rainfall, the latter species being most prevalent throughout the year. Populations of these two species were highest between December and July and lowest between August and October. Larval populations of Culex quinquefasciatus did not show a seasonal variation and larval populations of Cx. annulirostris were too low for any conclusions to be made. All species were found breeding most often in miscellaneous containers, with tyres, plant containers and flower vases also being important sources for Ae. aegypti breeding. Through **environmental** sanitation the Breteau Index for all species was reduced by 88%; Premise Index by 72% and the Container Index by 83%, when compared to a control area. ULV applied malathion was effective in temporarily reducing Ae. pseudoscutellaris populations from 50--100%. Effects on Ae. aegypti were inconclusive. It is concluded that through enforcement of the existing laws and strict monthly surveillance during the periods of highest seasonal density, urban Aedes and Culex populations can be maintained at an acceptable level.

L49 ANSWER 22 OF 23 MEDLINE on STN

80129498. PubMed ID: 7356398. Polypeptide synthesis in alphavirus-infected Aedes albopictus cells during the establishment of persistent infection. Richardson M A; Boulton R W; Raghow R S; Dalgarno L. Archives of virology, **(1980)** 63 (3-4) 263-74. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB Polypeptide synthesis was examined in mosquito cells during the establishment of a persistent infection with two alphaviruses, **Ross River virus** (RRV) and Semliki Forest virus (SFV), and in vertebrate cells cytopathically-infected with the same viruses. In Aedes albopictus cell, RRV reached peak titres at 34--48 hours p.i. At 12 hours 85 per cent of cells assayed as infected by infective centre assay; by 48 hours when persistence was established, virus production was reduced and less than 5 per cent of cells assayed as infected. There was no shut-down of host polypeptide synthesis during infection. Viral polypeptide synthesis was maximal between 10 and 24 hours p.i. The major viral polypeptides labelled were nucleocapsid protein and **envelope** protein(s). The precursor polypeptide p95 which was prominent in infected BHK cells was not detected in mosquito cells. Similar results were obtained on SFV infection. During the establishment of persistence there was a coordinate decline in the synthesis of RRV polypeptides, reaching undetectable levels by 72 hours p.i. Subculturing persitently-infected cells led to a small increase in viral polypeptide synthesis and virus titre. In contrast, during RRV growth in BHK celos host protein synthesis was severly inhibited and by 9--11 hours p.i. virus-specific polypeptide synthesis represented more than 90 per cent of total protein synthetic activity.

L49 ANSWER 23 OF 23 MEDLINE on STN

80086993. PubMed ID: 518301. Replication of standard and defective **Ross River virus** in BHK cells: patterns of viral RNA and polypeptide synthesis. Martin J H; Weir R C; Dalgarno L. Archives of virology, **(1979)** 61 (1-2) 87-103. Journal code: 7506870. ISSN: 0304-8608. Pub. country: Austria. Language: English.

AB Virus-specific macromolecule synthesis has been examined in BHK cells infected with **Ross River virus**. Unpassaged virus (R-0) and tenth-passage virus (R-10) have been compared. In infected cells R-0 generates i) 45S, 28S, 33S and 26S viral RNAs, ii) virus-specific precursor polypeptides of mol. wt. 127,000, 95,000 and 61,000 and iii) viral **envelope** proteins (mol. wts. 52,000 and 49,000) and nucleocapsid protein (mol. wt. 32,000). Thus in terms of virus-specific RNA and polypeptide synthesis, the replication of standard RRV is analogous to that of Semliki Forest virus and Sindbis virus. R-10 interferes with the replication of standard **Ross River virus** and generates large amounts of 19S and 24S defective RNA species; 45S and 26S RNA synthesis was not markedly affected. Defective RNAs are associated with RNAse-sensitive, 50S cytoplasmic particles which contain a variety of (mainly host) proteins but no nucleocapsid protein. No evidence for translation of defective RNAs was obtained. R-10 infection is also characterized by a

relatively early shut down of host protein synthesis and by a reduction in virus-specific polypeptide synthesis and nucleocapsid formation. The data suggest that defective **Ross River virus** interferes primarily at the translational level.

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FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
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L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

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E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
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L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
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L19 3 S E3
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FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21 13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25 21 S L24 AND AY<2000
L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000
L31 80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32 16 S L31 AND (MARKER?/CLM)

```
L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/TI)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38      123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39        1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40          85 S L39 AND (GAG AND POL)
L41           0 S L40 AND (MULTIVALENT)
L42           7 S L40 AND MARKER?
L43          67 S L40 AND PY<2000
L44         602 S L38 AND (LENTIVIR? EXPRESSION VECTOR? OR LENTIVIR? VECTOR?)
L45          65 S L44 AND PY<2000
L46           8 S L45 AND (MARKER? OR SELECTABLE OR DETECTABLE)
L47         269 S (ROSS RIVER VIRUS OR ROSS RIVER ALPHAVIRUS)
L48          39 S L47 AND (ENV?)
L49          23 S L48 AND PY<1999

=> e richman d/au
E1            2     RICHMAN CAROL M/AU
E2            1     RICHMAN CHARMAINE/AU
E3          109 --> RICHMAN D/AU
E4            3     RICHMAN D A/AU
E5          305     RICHMAN D D/AU
E6            2     RICHMAN D J/AU
E7            3     RICHMAN D L/AU
E8            8     RICHMAN D M/AU
E9            1     RICHMAN D N/AU
E10          69     RICHMAN D P/AU
E11           3     RICHMAN D W/AU
E12           2     RICHMAN DAVID M/AU

=> e richman douglas ?/au
E1            4     RICHMAN DAVID P/AU
E2            7     RICHMAN DOUGLAS/AU
E3            0 --> RICHMAN DOUGLAS ?/AU
E4           21     RICHMAN DOUGLAS D/AU
E5            6     RICHMAN E/AU
E6            3     RICHMAN E A/AU
E7            1     RICHMAN E E/AU
E8            1     RICHMAN E I/AU
E9            3     RICHMAN E M/AU
E10           3     RICHMAN EISENSTAT J/AU
E11           4     RICHMAN EISENSTAT J B/AU
E12           3     RICHMAN F/AU

=> s e4
L50          21 "RICHMAN DOUGLAS D"/AU

=> d l50,ti,1-21

L50  ANSWER 1 OF 21     MEDLINE on STN
TI   Genetic basis of hypersusceptibility to protease inhibitors and low
     replicative capacity of human immunodeficiency virus type 1 strains in
     primary infection.

L50  ANSWER 2 OF 21     MEDLINE on STN
TI   Nucleoside and nucleotide analogue reverse transcriptase inhibitors: a
     clinical review of antiretroviral resistance.

L50  ANSWER 3 OF 21     MEDLINE on STN
TI   Drug resistance mutations in HIV-1.

L50  ANSWER 4 OF 21     MEDLINE on STN
```

TI A multi-investigator, international DNA bank for AIDS related human genetic studies: AACTG Protocol A5128.

L50 ANSWER 5 OF 21 MEDLINE on STN
TI Broadly increased sensitivity to cytotoxic T lymphocytes resulting from Nef epitope escape mutations.

L50 ANSWER 6 OF 21 MEDLINE on STN
TI Questions to and answers from the International AIDS Society-USA Resistance Testing Guidelines Panel.

L50 ANSWER 7 OF 21 MEDLINE on STN
TI Drug resistance mutations in HIV-1.

L50 ANSWER 8 OF 21 MEDLINE on STN
TI Antiretroviral drug resistance testing in adults infected with human immunodeficiency virus type 1: 2003 recommendations of an International AIDS Society-USA Panel.

L50 ANSWER 9 OF 21 MEDLINE on STN
TI Polyclonal proliferation and apoptosis of CCR5+ T lymphocytes during primary human immunodeficiency virus type 1 infection: regulation by interleukin (IL)-2, IL-15, and Bcl-2.

L50 ANSWER 10 OF 21 MEDLINE on STN
TI Clade B HIV-1 superinfection with wild-type virus after primary infection with drug-resistant clade B virus.

L50 ANSWER 11 OF 21 MEDLINE on STN
TI Rapid evolution of the neutralizing antibody response to HIV type 1 infection.

L50 ANSWER 12 OF 21 MEDLINE on STN
TI Transmission fitness of drug-resistant human immunodeficiency virus and the prevalence of resistance in the antiretroviral-treated population.

L50 ANSWER 13 OF 21 MEDLINE on STN
TI Multiple measures of HIV burden in blood and tissue are correlated with each other but not with clinical parameters in aviremic subjects.

L50 ANSWER 14 OF 21 MEDLINE on STN
TI Baseline predictors of CD4 T-lymphocyte recovery with combination antiretroviral therapy.

L50 ANSWER 15 OF 21 MEDLINE on STN
TI Antiretroviral-drug resistance among patients recently infected with HIV.

L50 ANSWER 16 OF 21 MEDLINE on STN
TI Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel.

L50 ANSWER 17 OF 21 MEDLINE on STN
TI Effects of antiretroviral drugs on human immunodeficiency virus type 1-induced CD4(+) T-cell death.

L50 ANSWER 18 OF 21 MEDLINE on STN
TI A phase II randomized study of HIV-specific T-cell gene therapy in subjects with undetectable plasma viremia on combination antiretroviral therapy.

L50 ANSWER 19 OF 21 MEDLINE on STN
TI Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections.

L50 ANSWER 20 OF 21 MEDLINE on STN
TI Dynamics of T cell responses in HIV infection.

```
L50  ANSWER 21 OF 21     MEDLINE on STN
TI   Persistent HIV-1-specific cellular responses despite prolonged therapeutic
     viral suppression.


=> e jolly douglas ?/au
E1            1     JOLLY DOMINIQUE/AU
E2            3     JOLLY DONALD T/AU
E3            0 --> JOLLY DOUGLAS ?/AU
E4            2     JOLLY DOUGLAS J/AU
E5           13     JOLLY E/AU
E6            6     JOLLY E C/AU
E7            5     JOLLY E E/AU
E8            3     JOLLY E J/AU
E9            4     JOLLY E R/AU
E10           1     JOLLY ELAINE/AU
E11           2     JOLLY ELAINE E/AU
E12          25     JOLLY G/AU

=> s e4
L51           2 "JOLLY DOUGLAS J"/AU

=> d l51,ti,1-2

L51  ANSWER 1 OF 2     MEDLINE on STN
TI   Phase 1 trial of FVIII gene transfer for severe hemophilia A using a
     retroviral construct administered by peripheral intravenous infusion.

L51  ANSWER 2 OF 2     MEDLINE on STN
TI   Purified herpes simplex virus thymidine kinase retroviral particles: III.
     Characterization of bystander killing mechanisms in transfected tumor
     cells.


=> e jolly d j/au
E1           16     JOLLY D E/AU
E2           10     JOLLY D H/AU
E3           68 --> JOLLY D J/AU
E4            2     JOLLY D L/AU
E5            1     JOLLY D M/AU
E6           11     JOLLY D T/AU
E7           19     JOLLY D W/AU
E8            2     JOLLY DAMIEN/AU
E9            2     JOLLY DAVID/AU
E10           1     JOLLY DAVID G/AU
E11           1     JOLLY DESMOND A/AU
E12           1     JOLLY DOMINIQUE/AU

=> s e3
L52          68 "JOLLY D J"/AU

=> s l52 and py<1999
      11672474 PY<1999
L53          50 L52 AND PY<1999

=> d l53,ti,1-10

L53  ANSWER 1 OF 50     MEDLINE on STN
TI   Influence of cell polarity on retrovirus-mediated gene transfer to
     differentiated human airway epithelia.

L53  ANSWER 2 OF 50     MEDLINE on STN
TI   Effects of keratinocyte and hepatocyte growth factor in vivo: implications
     for retrovirus-mediated gene transfer to liver.
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L53 ANSWER 3 OF 50 MEDLINE on STN
TI Genetic immunization of chimpanzees chronically infected with the hepatitis B virus, using a recombinant retroviral vector encoding the hepatitis B virus core antigen.

L53 ANSWER 4 OF 50 MEDLINE on STN
TI Use of a recombinant murine cytomegalovirus expressing vesicular stomatitis virus G protein to pseudotype retroviral vectors.

L53 ANSWER 5 OF 50 MEDLINE on STN
TI Direct synovial gene transfer with retroviral vectors in rat adjuvant arthritis.

L53 ANSWER 6 OF 50 MEDLINE on STN
TI Retroviral-mediated expression of FIV envelope/Rev induces CD8+ CTL responses in mice.

L53 ANSWER 7 OF 50 MEDLINE on STN
TI Human immunodeficiency virus immunotherapy using a retroviral vector.

L53 ANSWER 8 OF 50 MEDLINE on STN
TI Anti-vector immunoglobulin induced by retroviral vectors.

L53 ANSWER 9 OF 50 MEDLINE on STN
TI Evaluation of PCR and ELISA assays for screening clinical trial subjects for replication-competent retrovirus.

L53 ANSWER 10 OF 50 MEDLINE on STN
TI Alveolar macrophages inhibit retrovirus-mediated gene transfer to airway epithelia.

=> d l53,cbib,ab,1-50

L53 ANSWER 1 OF 50 MEDLINE on STN
1999030889. PubMed ID: 9811717. Influence of cell polarity on retrovirus-mediated gene transfer to differentiated human airway epithelia. Wang G; Davidson B L; Melchert P; Slepushkin V A; van Es H H; Bodner M; **Jolly D J**; McCray P B Jr. (Departments of Pediatrics, University of Iowa College of Medicine, Iowa City, Iowa 52242, USA.) Journal of virology, **(1998 Dec)** 72 (12) 9818-26. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.
AB Gene transfer with recombinant murine leukemia viruses (MuLV) provides the potential to permanently correct inherited lung diseases, such as cystic fibrosis (CF). Several problems prevent the application of MuLV-based recombinant retroviruses to lung gene therapy: (i) the lack of cell proliferation in mature pulmonary epithelia, (ii) inefficient gene transfer with a vector applied to the apical surface, and (iii) low titers of many retroviral preparations. We found that keratinocyte growth factor (KGF) stimulated proliferation of differentiated human tracheal and bronchial epithelia. Approximately 50% of epithelia divided in response to KGF as assessed by bromodeoxyuridine histochemistry. In airway epithelia stimulated to divide with KGF, high-titer ampho- and xenotropic enveloped vectors preferentially infected cells from the basal side. However, treatment with hypotonic shock or EGTA transiently increased transepithelial permeability, enhancing gene transfer with the vector applied to the mucosal surfaces of KGF-stimulated epithelia. Up to 35% of cells expressed the transgene after gene transfer. By using this approach, cells throughout the epithelial sheet, including basal cells, were targeted. Moreover, the Cl- transport defect in differentiated CF airway epithelia was corrected. These findings suggest that barriers to apical infection with MuLV can be overcome.

L53 ANSWER 2 OF 50 MEDLINE on STN
1998386039. PubMed ID: 9721085. Effects of keratinocyte and hepatocyte growth factor in vivo: implications for retrovirus-mediated gene transfer

Es H H; Nakamura T; Matsumoto K; Davidson B L. (Department of Internal Medicine, University of Iowa College of Medicine, Iowa City 52242, USA.) Human gene therapy, **(1998 Aug 10)** 9 (12) 1747-54. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB We have previously shown that intravenous administration of keratinocyte growth factor (KGF) induces hepatocyte proliferation, allowing for efficient and noninvasive in vivo gene transfer with high-titer retroviral vectors in mice. The distinctive periportal distribution of transduced cells led us to investigate the ability of virus-sized particles to perfuse the liver adequately after growth factor treatment. We found that perfusion was adequate, and that transduction was limited to the periportal region because only those cells were stimulated to divide. Cells in this region also showed increased expression of Ram-1, the receptor for the murine Moloney leukemia virus (MoMLV) amphotropic envelope, after KGF treatment. In further studies we found that recombinant hepatocyte growth factor (HGF) induces a different population of hepatocytes to divide and upregulate Ram-1. The differential pattern of induction suggested that combining KGF and HGF would improve gene transfer efficiency further. Indeed, simultaneous delivery of both growth factors leads to an overall increase in the number of proliferating cells. Importantly, when coupled with MoMLV delivery, efficiency of gene transfer increased. These results confirm the utility of growth factors for noninvasive hepatic gene transfer in mice, and demonstrate how experiments to define the mechanism of transduction can be taken advantage of to develop improved gene transfer protocols.

L53 ANSWER 3 OF 50 MEDLINE on STN

1998386036. PubMed ID: 9721082. Genetic immunization of chimpanzees chronically infected with the hepatitis B virus, using a recombinant retroviral vector encoding the hepatitis B virus core antigen. Sallberg M; Hughes J; Javadian A; Ronlov G; Hultgren C; Townsend K; Anderson C G; O'Dea J; Alfonso J; Eason R; Murthy K K; **Jolly D J**; Chang S M; Mento S J; Milich D; Lee W T. (Chiron Technologies Center for Gene Therapy, San Diego, CA 92121-1204, USA.) Human gene therapy, **(1998 Aug 10)** 9 (12) 1719-29. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Cytotoxic T lymphocyte (CTL) activity and CD4+ helper T cell responses to the hepatitis B virus (HBV) core antigen (HBcAg) have been implicated in clearance of acute and chronic HBV infections. We showed that intramuscular injections of a novel recombinant retroviral vector expressing an HBcAg-neomycin phosphotransferase II (HBc-NEO) fusion protein induces HBc/eAg-specific antibodies and CD4+ and CD8+ T cell responses in mice and rhesus monkeys. We have now immunized three chronically infected chimpanzees, each with 10(10) CFU of nonreplicating retroviral vector particles expressing the HBc-NEO fusion protein. Of two immunized chimpanzees examined for CTL responses, one developed HBcAg-specific CTLs and showed marginal, transient elevations of alanine aminotransferase (ALT) levels following injection. However, both chimpanzees remained positive for serum HBeAg, negative for anti-HBe antibody by conventional assays, and displayed no change in HBV viral load throughout the study. In contrast, the third chimpanzee exhibited a traditional seroconversion evidenced by a loss of serum HBeAg and the subsequent emergence of anti-HBe antibodies within 24 weeks after the first injection. Simultaneously, two transient ALT flares and a significant decrease in the serum HBV DNA levels were noted. Despite its limitations, the present study demonstrates (1) the safety of treatment with high titers of retroviral vector in chimpanzees, (2) the capability of a retroviral vector expressing HBcAg to stimulate immune responses in HBV chronic carrier chimpanzees, and (3) that retroviral vector immunization may be therapeutically beneficial in the treatment of chronic HBV infection.

L53 ANSWER 4 OF 50 MEDLINE on STN

1998368870. PubMed ID: 9705172. Use of a recombinant murine cytomegalovirus expressing vesicular stomatitis virus G protein to

pseudotype retroviral vectors. Manning W C, Ralph S J, **Jolly D J**, Nemes S J; Ralston R O. (Chiron Corporation, Emeryville, CA 94608, USA.. william_manning@cc.chiron.com) . Journal of virological methods, **(1998 Jul)** 73 (1) 31-9. Journal code: 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A new method of producing vesicular stomatitis virus (VSV) G protein pseudotyped retroviral vectors is described. In this method, stocks of VSV-G pseudotyped vector were reproducibly obtained by infecting an env-, human, retroviral vector producer cell line with a recombinant murine cytomegalovirus (CMV) which expresses VSV-G protein. The recombinant murine CMV, RMCMVG, expressed VSV-G protein under transcriptional control of the human CMV immediate-early promoter. RMCMVG, like murine CMV, can infect human cells, but the infection is limited to the expression of the viral immediate-early genes; no productive replication of murine CMV occurs. Recombinant murine CMV vector infection of non-permissive cells may be useful in situations where high levels of gene expression are desired without concomitant viral vector replication.

L53 ANSWER 5 OF 50 MEDLINE on STN

1998293835. PubMed ID: 9632074. Direct synovial gene transfer with retroviral vectors in rat adjuvant arthritis. Nguyen K H; Boyle D L; McCormack J E; Chada S; **Jolly D J**; Firestein G S. (Division of Rheumatology, University of California, San Diego, School of Medicine, La Jolla 92093-0656, USA.) Journal of rheumatology, **(1998 Jun)** 25 (6) 1118-25. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE: To evaluate the feasibility of direct in vivo gene transfer in an animal model of arthritis using a retroviral vector. METHODS: The timing and dose of retroviral vector was examined using very high titer retroviral vector (> or = 10(9) CFU) in rat adjuvant arthritis. Retroviral vector expressing beta-galactosidase (beta-gal) or vehicle alone was injected into the right ankle of rats with adjuvant arthritis. Ankles were injected either on Day 7 (pre-arthritis), Day 10 (early arthritis), Day 15 (accelerating arthritis), or Day 28 (chronic arthritis) after adjuvant immunization. Joints were harvested 3 days later and extracts were assayed for beta-gal activity. RESULTS: Synovial beta-gal expression was minimal in the Day 7 group and elevated in the Day 10, Day 15, and Day 28 groups. Gene transfer with retroviral vector did not exacerbate the local inflammatory response. Minimal or no beta-gal expression was observed in the contralateral uninjected paw or in the spleen, lung, liver, and kidneys. Frozen sections of retroviral vector injected joints were stained with X-gal and revealed transduced cells in the lining and superficial sublining layers. To determine the longevity of gene expression, ankle joints were injected with vector on Day 15 post-adjuvant, harvested, and assayed for beta-gal activity for up to 49 days after injection. Expression of the enzyme peaked from Day 3 to 7 and was still readily detected up to 49 days after retrovirus infection. CONCLUSION: This is the first report of successful direct in vivo gene transfer in the rat adjuvant arthritis model using a retroviral vector. Appropriate timing of administration and very high titer retroviral vector preparations are key determinants of adequate gene transduction.

L53 ANSWER 6 OF 50 MEDLINE on STN

1998275676. PubMed ID: 9612729. Retroviral-mediated expression of FIV envelope/Rev induces CD8+ CTL responses in mice. von Schwedler U; Townsend K; Chada S; **Jolly D J**; Elder J; Chang S M; Lee W T. (Chiron Technologies Center for Gene Therapy, San Diego, Calif. 92121, USA.) Intervirology, **(1997)** 40 (4) 271-6. Journal code: 0364265. ISSN: 0300-5526. Pub. country: Switzerland. Language: English.

AB Recombinant retroviral vectors that express the Env and Rev proteins of feline immunodeficiency virus (FIV) were prepared and analyzed in a mouse model system for their ability to induce antigen-specific CD8+ CTL (cytotoxic T lymphocyte) responses. The ultimate goal of these studies is to develop effective immunogens for CTL induction in the cat. Recombinant Env/Rev retroviral vectors were used to transduce mouse fibroblasts and these cells were then inoculated intraperitoneally into syngeneic BALB/c

of recombinant retroviral vector-transduced cells to elicit cell-mediated immunity in the mouse model offers the possibility that such delivery systems may serve as therapeutic and/or prophylactic treatments against FIV infection in the cat.

L53 ANSWER 7 OF 50 MEDLINE on STN
1998140469. PubMed ID: 9479840. Human immunodeficiency virus immunotherapy using a retroviral vector. Warner J F; **Jolly D J**; Merritt J. (Chiron Viagene, San Diego, CA 92121, USA.) Current topics in microbiology and immunology, **(1998)** 226 145-60. Ref: 40. Journal code: 0110513. ISSN: 0070-217X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L53 ANSWER 8 OF 50 MEDLINE on STN
97358647. PubMed ID: 9215743. Anti-vector immunoglobulin induced by retroviral vectors. McCormack J E; Martineau D; DePolo N; Maifert S; Akbarian L; Townsend K; Lee W; Irwin M; Sajjadi N; **Jolly D J**; Warner J. (Chiron Technologies, Center for Gene Therapy, San Diego, CA 92121, USA.) Human gene therapy, **(1997 Jul 1)** 8 (10) 1263-73. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.
AB Replication-incompetent retroviruses have been employed as gene therapy vectors in experimental settings for more than a decade. More recently, these vectors have been tested in the clinic as immunotherapeutic agents and anticancer agents. One potential problem with the use of such vectors is the possible development of immune responses directed against the vector particles themselves. Here, we examine immunoglobulin (Ig) responses specific for retroviral vectors derived from murine leukemia virus (MLV). Anti-MLV Ig is seen following intramuscular (i.m.) administration of retroviral vectors in mice, and in nonhuman primates; as expected, these responses are dependent upon the vector dose delivered. Furthermore, serum from vector-treated animals is capable of partially neutralizing vector-mediated transduction of target cells in an in vitro assay. Nevertheless, even in the presence of significant levels of anti-vector Ig in vivo, i.m. administration of retroviral vector is still capable of driving both Ig and cytotoxic T lymphocyte (CTL) responses specific for vector-encoded gene products. This work suggests that although retroviral vectors may readily induce immune responses directed against the vector particles themselves, such responses will not significantly affect the efficiency of these vectors in an immunotherapeutic protocol.

L53 ANSWER 9 OF 50 MEDLINE on STN
97358644. PubMed ID: 9215740. Evaluation of PCR and ELISA assays for screening clinical trial subjects for replication-competent retrovirus. Martineau D; Klump W M; McCormack J E; DePolo N J; Kamantigue E; Petrowski M; Hanlon J; **Jolly D J**; Mento S J; Sajjadi N. (Chiron Technologies, Center for Gene Therapy, San Diego, CA 92121-1204, USA.) Human gene therapy, **(1997 Jul 1)** 8 (10) 1231-41. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.
AB Gene delivery via murine-based recombinant retroviral vectors is currently widely used in gene therapy clinical trials. The vectors are engineered to be replication defective by replacing the structural and nonstructural genes of a cloned infectious retrovirus with a therapeutic gene of interest. The retroviral particles are currently generated in packaging cell lines, which supply all retroviral proteins in trans. Recombination between short homologous regions of the retroviral vector and packaging cell line elements can theoretically generate replication-competent retrovirus (RCR) and hence the Food and Drug Administration (FDA) requires the monitoring of clinical trial subjects for the presence of RCR. Sensitive polymerase chain reaction (PCR) assays have been used for the detection of murine leukemia virus (MLV) nucleotide sequences in peripheral blood mononuclear cells (PBMCs). A novel serological enzyme-linked immunosorbent assay (ELISA) for the detection of anti-MLV specific immunoglobulin (Ig) has been developed to be used as an alternative to the PCR assay. Both assays were used to monitor human

immunodeficiency virus (HIV) positive clinical trial subjects who had received multiple injections of HIV-IT (V), a retroviral vector encoding HIV-1 IIIBenv/rev. Western blot analysis and an in vitro vector neutralization assay were used to characterize further a subset of serum samples tested by ELISA. Results show no evidence of RCR infection in clinical trial subjects. PCR and ELISA assays are discussed in terms of their advantages and limitations as routine screening assays for RCR. The PCR assay is our current choice for monitoring clinical trial subjects receiving direct administration of vector, and the ELISA is our choice for those receiving ex vivo treatment regimens.

L53 ANSWER 10 OF 50 MEDLINE on STN

97333606. PubMed ID: 9189766. Alveolar macrophages inhibit retrovirus-mediated gene transfer to airway epithelia. McCray P B Jr; Wang G; Kline J N; Zabner J; Chada S; **Jolly D J**; Chang S M; Davidson B L. (Department of Pediatric, University of Iowa College of Medicine, Iowa City 52242.) Human gene therapy, **(1997 Jun 10)** 8 (9) 1087-93. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Gene transfer with integrating vectors such as recombinant retrovirus has the potential to correct inherited lung diseases permanently. As a gene therapy target, the pulmonary epithelium presents several challenges to vector delivery in vivo. Many of the host defenses that have evolved to prevent infection from inhaled bacteria or viruses represent potential barriers to gene transfer to the lung. We performed in vitro studies to determine whether two components of the innate immune system of the lung, airway surface fluid and alveolar macrophages, inhibit retroviral gene transfer to airway epithelia. Human alveolar macrophages obtained by bronchoalveolar lavage from normal subjects were left untreated or activated with lipopolysaccharide (LPS) for 3 hr in the presence of subconfluent human bronchial epithelial cells (HBE); than 4 x 10(5) cfu DA-luciferase retrovirus was added. Three days after infection, luciferase activity was measured in cell lysates. When the epithelial cells were co-cultured with LPS-activated macrophages, retroviral gene transfer to HBE cells was reduced by approximately 60%. Nonactivated macrophages decreased the transfection to approximately 55% of control values. In control experiments with either activated or inactivated macrophages but without epithelia, no luciferase activity was detected, suggesting that terminally differentiated alveolar macrophages are not infected by the recombinant retrovirus. Pretreatment of alveolar macrophages with dexamethasone restored gene transfer to approximately 60% of control values. In contrast, incubation of retrovirus with airway surface fluid had no inhibitory effect on gene transfer. These experiments document that AM inhibit retrovirus-mediated gene transfer to airway epithelia in vitro, and may represent a barrier to retroviral gene transfer in vivo. These barriers may be overcome, at least partially, with pharmacological agents.

L53 ANSWER 11 OF 50 MEDLINE on STN

97332364. PubMed ID: 9188598. Characterization of humoral and CD4+ cellular responses after genetic immunization with retroviral vectors expressing different forms of the hepatitis B virus core and e antigens. Sallberg M; Townsend K; Chen M; O'Dea J; Banks T; **Jolly D J**; Chang S M; Lee W T; Milich D R. (Department of Molecular Biology, Scripps Research Institute, La Jolla, California 92037, USA.. masa@vird01.hs.sll.se) . Journal of virology, **(1997 Jul)** 71 (7) 5295-303. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The humoral and CD4+ cellular immune responses in mice following genetic immunization with three retroviral vectors encoding different forms of hepatitis B virus core antigen (HBcAg) and e antigen (HBeAg) were analyzed. The retroviral vectors induced expression of intracellular HBcAg (HBc[3A4]), secreted HBeAg (HBe[5A2]), or an intracellular HBcAg-neomycin phosphoryltransferase fusion protein (HBc-NEO[6A3]). Specific antibody levels and immunoglobulin G isotype restriction were highly dependent on both the host major histocompatibility complex and the transferred gene. Humoral and CD4+ cellular HBcAg and/or HBeAg

(HBc/eAg)-specific immune responses following retroviral vector immunization were of a lower magnitude but followed the same characteristics compared with those after immunization with HBc/eAg in adjuvant. Two factors influenced the humoral responses. First, in vivo depletion of CD8+ cells in HBc-NEO[6A3]-immunized H-2k mice abrogated both HBcAg-specific antibodies and in vitro-detectable cytotoxic T lymphocytes. Second, priming of H-2b mice with an HBc/eAg-derived T-helper (Th) peptide in adjuvant prior to retroviral vector immunization greatly enhanced the HBc/eAg-specific humoral responses to all three vectors, suggesting that insufficient HBc/eAg-specific CD4+ Th-cell priming limits the humoral responses. In conclusion, direct injection of retroviral vectors seems to be effective in priming HBc/eAg-specific CD8+ but comparatively inefficient in priming CD4+ Th cells and subsequently specific antibodies. However, the limited HBc/eAg-specific CD4+ cell priming can effectively be circumvented by prior administration of a recombinant or synthetic form of HBc/eAg in adjuvant.

L53 ANSWER 12 OF 50 MEDLINE on STN

97248377. PubMed ID: 9094605. Characterization of CD8+ cytotoxic T-lymphocyte responses after genetic immunization with retrovirus vectors expressing different forms of the hepatitis B virus core and e antigens. Townsend K; Sallberg M; O'Dea J; Banks T; Driver D; Sauter S; Chang S M; **Jolly D J**; Mento S J; Milich D R; Lee W T. (Chiron Technologies Center for Gene Therapy, San Diego, California 92121, USA.) Journal of virology, **(1997 May)** 71 (5) 3365-74. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Cytotoxic T-lymphocyte (CTL) activity appears to play an important role in resolving hepatitis B virus (HBV) infection, and the ability to induce such responses remains an important goal for developing effective immunotherapeutics. A panel of recombinant retrovirus vectors expressing different forms of the HBV core antigen (HBcAg) or e antigen (eAg) were found to induce antigen-specific major histocompatibility complex-restricted CTL responses in both mice and macaques. In addition, a novel retrovirus vector expressing an HBcAg-neomycin phosphotransferase II (HBc-Neo) fusion protein [LHBc-NEO(6A3)], which allows the measurement of the anti-Neo antibody response as a means of directly tracking biological activity of the vector, was generated. Doses greater than 10(7) CFU were necessary to induce CTL responses in H-2(k) mice. Intramuscular injections with 10(8) CFU of the LHBc-NEO(6A3) retrovirus vector into rhesus monkeys induced HBc/eAg-specific antibody production and CD8+ CTLs. The CTL response from one of the two responder rhesus monkeys was directed against a 9-residue peptide, GELMTLATW, at positions 63 to 71 of the HBc/eAg sequence. The CTL response is long lived, being detectable as late as 16 weeks after immunization, and can be boosted upon reimmunization. The potent ability of recombinant retrovirus vectors to induce HBcAg- and eAg-specific CTL responses may prove beneficial as a therapeutic treatment for chronic hepatitis B infection.

L53 ANSWER 13 OF 50 MEDLINE on STN

97203166. PubMed ID: 9050884. Antigen presentation in retroviral vector-mediated gene transfer in vivo. Song E S; Lee V; Surh C D; Lynn A; Brumm D; **Jolly D J**; Warner J F; Chada S. (Department of Immunobiology, Chiron Technology-Center for Gene Therapy, San Diego, CA 92121, USA.. elizabeth_song@cc.chiron.com) . Proceedings of the National Academy of Sciences of the United States of America, **(1997 Mar 4)** 94 (5) 1943-8. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have examined mechanisms involved in gene transfer, protein expression, and antigen presentation after direct administration of retroviral vectors using a variety of antigen systems. We have identified transduced infiltrating cells at the injection site, and the majority of the infiltrating cells were of the monocyte/macrophage lineage. We found that the splenic dendritic cell fraction contained proviral DNA, expressed antigenic proteins, and was able to present antigens efficiently to the immune system. Furthermore, the dendritic cell fractions from retroviral vector-immunized mice were able to prime naive T cells in vitro, and

adoptive transfer of in vitro stimulated dendritic cell nucleus elicited antigen-specific cytotoxic T lymphocytes. These data suggest a role for dendritic cells in induction of immune responses elicited by retroviral vector-mediated gene transfer.

L53 ANSWER 14 OF 50 MEDLINE on STN

97136510. PubMed ID: 8981911. Proliferation induced by keratinocyte growth factor enhances in vivo retroviral-mediated gene transfer to mouse hepatocytes. Bosch A; McCray P B Jr; Chang S M; Ulich T R; Simonet W S; **Jolly D J**; Davidson B L. (Department of Internal Medicine, University of Iowa, Iowa City 52242, USA.) Journal of clinical investigation, **(1996 Dec 15)** 98 (12) 2683-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB Retroviral gene transfer to liver without prior injury has not yet been accomplished. We hypothesized that recombinant human keratinocyte growth factor would stimulate proliferation of hepatocytes and allow for efficient in vivo gene transfer with high titer murine Moloney retroviral vectors. This report shows that 48 h after intravenous injection of keratinocyte growth factor, hepatocyte proliferation increased approximately 40-fold compared to non-stimulated livers. When keratinocyte growth factor treatment was followed by intravenous injection of high titer (1 x 10(8) colony forming units/ml) retrovirus coding for the Escherichia Coli beta-galactosidase gene, there was a 600-fold increase in beta-galactosidase expression, with 2% of hepatocytes transduced. Thus, by exploiting the mitogenic properties of keratinocyte growth factor, retrovirus-mediated gene transfer to liver may be accomplished in vivo without the use of partial hepatectomy or pretreatment with other toxins to induce hepatocyte cell division.

L53 ANSWER 15 OF 50 MEDLINE on STN

96135369. PubMed ID: 8546403. Layered amplification of gene expression with a DNA gene delivery system. Driver D A; Latham E M; Polo J M; Belli B A; Banks T A; Chada S; Brumm D; Chang S M; Mento S J; **Jolly D J**; +. (Viagene Inc., San Diego, California 92121, USA.) Annals of the New York Academy of Sciences, **(1995 Nov 27)** 772 261-4. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

L53 ANSWER 16 OF 50 MEDLINE on STN

96099465. PubMed ID: 8523564. Sindbis virus DNA-based expression vectors: utility for in vitro and in vivo gene transfer. Dubensky T W Jr; Driver D A; Polo J M; Belli B A; Latham E M; Ibanez C E; Chada S; Brumm D; Banks T A; Mento S J; **Jolly D J**; Chang S M. (Department of Viral and Genetic Therapeutics, Viagene, Inc., San Diego, California 92121, USA.) Journal of virology, **(1996 Jan)** 70 (1) 508-19. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Several DNA-based Sindbis virus vectors were constructed to investigate the feasibility and potential applications for initiating the virus life cycle in cells transfected directly with plasmid DNA. These vectors, when transfected into mammalian cells, have been used to produce virus, to express heterologous genes, and to produce infectious vector particles. This approach involved the conversion of a self-replicating vector RNA (replicon) into a layered DNA-based expression system. The first layer includes a eukaryotic RNA polymerase II expression cassette that initiates nuclear transcription of an RNA which corresponds to the Sindbis virus vector replicon. Following transport of this RNA from the nucleus to the cytoplasm, the second layer, autocatalytic amplification of the vector, proceeds according to the Sindbis virus replication cycle and results in expression of the heterologous gene. The Sindbis virus DNA vectors expressed reporter genes in transfected cells at levels that were comparable to those of in vitro-transcribed RNA replicons and were approximately 10-fold higher than the levels produced by conventional RNA polymerase II-dependent plasmids in which the promoter and reporter gene were linked directly. Reporter gene expression was also observed in rodent muscle following injection with Sindbis virus DNA vectors. In a second application, packaged vector particles were produced in cells cotransfected with complementing replicon and defective helper DNAs. The

alphavirus-based vector systems in general and also provide a vector with broad potential applications for genetic immunization.

L53 ANSWER 17 OF 50 MEDLINE on STN
95200571. PubMed ID: 7893440. Cytotoxic T-lymphocyte induction in asymptomatic HIV-1-infected patients immunized with Retrovector-transduced autologous fibroblasts expressing HIV-1IIIB Env/Rev proteins. Ziegner U H; Peters G; **Jolly D J**; Mento S J; Galpin J; Prussak C E; Barber J R; Hartnett D E; Bohart C; Klump W; +. (Viagene, Inc., San Diego, CA 92121.) AIDS (London, England), **(1995 Jan)** 9 (1) 43-50. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: To demonstrate the safety and enhancement of HIV-1-specific immune responses in HIV-infected asymptomatic patients following treatment with retroviral vector (Retrovector)-transduced autologous fibroblasts (VTAF) expressing HIV-1IIIB Env/Rev proteins. DESIGN: A non-placebo-controlled, single arm Phase I study. PARTICIPANTS: Four HIV-1-seropositive asymptomatic volunteers were selected based on age (18-50 years), CD4/CD3 lymphocyte counts (> 600 x 10(6)/l or > 40%), and positive delayed-type hypersensitivity test to at least one recall antigen. INTERVENTIONS: Patients were treated at 2-week intervals with a total of three intramuscular injections of irradiated autologous fibroblasts transduced with a molecularly engineered, non-replicating amphotropic murine retrovector encoding the HIV-1IIIB Env/Rev proteins. MAIN OUTCOME MEASURES: The clinical status of patients was assessed by history, physical examination, serum chemistry and hematology, CD4/CD3 lymphocyte counts, HIV viral burden, and monitored throughout the study to detect potentially treatment-induced toxic or unwanted side-effects. In addition, HIV-1-specific cytotoxic T-lymphocyte (CTL) activity was measured to determine the biological activity of VTAF. RESULTS: No acute local or systemic adverse events occurred following three injections with VTAF. Furthermore, a statistically significant increase of CD8+ CTL activity against HIV-1IIIB Env/Rev-expressing targets was observed in peripheral blood mononuclear cells from two out of four patients. CONCLUSIONS: This is the first report of the administration of a gene transfer treatment to HIV-1-infected patients and provides initial support for the safety and activity of retrovector-transduced fibroblasts administered to asymptomatic patients. This treatment resulted in the detection of increased HIV-1IIIB Env/Rev-specific CTL activity in two HIV-seropositive patients and could provide a better understanding of the role of CTL activity in HIV disease progression.

L53 ANSWER 18 OF 50 MEDLINE on STN
94309169. PubMed ID: 8035504. Direct injection of a recombinant retroviral vector induces human immunodeficiency virus-specific immune responses in mice and nonhuman primates. Irwin M J; Laube L S; Lee V; Austin M; Chada S; Anderson C G; Townsend K; **Jolly D J**; Warner J F. (Department of Immunobiology, Viagene, Inc., San Diego, California 92121.) Journal of virology, **(1994 Aug)** 68 (8) 5036-44. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The cytotoxic T-lymphocyte (CTL) response plays an important role in controlling the severity and duration of viral infections. Immunization by direct in vivo administration of retroviral vector particles represents an efficient means of introducing and expressing genes and, subsequently, the proteins they encode in vivo in mammalian cells. In this manner foreign proteins can be provided to the endogenous, class I major histocompatibility complex antigen presentation pathway leading to CTL activation. A nonreplicating recombinant retroviral vector, encoding the human immunodeficiency virus type 1 (HIV-1) IIIB envelope and rev proteins, has been developed and examined for stimulation of immune responses in mouse, rhesus macaque, and baboon models. Animals were immunized by direct intramuscular injection of the retroviral vector particles. Vector-immunized mice, macaques, and baboons generated long-lived CD8+, major histocompatibility complex-restricted CTL responses that were HIV-1 protein specific. The CTL responses were found to be dependent on the ability of the retroviral vector to transduce cells. The

and baboons. These studies demonstrate the ability of a retroviral vector encoding HIV-1 proteins to stimulate cellular and humoral immune responses and suggest that retrovector immunization may provide an effective means of inducing or augmenting CTL responses in HIV-1-infected individuals.

L53 ANSWER 19 OF 50 MEDLINE on STN

94295989. PubMed ID: 8024193. Retrovirus-mediated gene transfer of the human gamma-IFN gene: a therapy for cancer. Howard B; Burrascano M; McCallister T; Chong K; Gangavalli R; Severinsson L; **Jolly D J**; Darrow T; Vervaert C; Abdel-Wahab Z; +. (Viagene, Inc., San Diego, California 92121.) Annals of the New York Academy of Sciences, **(1994 May 31)** 716 167-87. Journal code: 7506858. ISSN: 0077-8923. Pub. country: United States. Language: English.

AB A retroviral vector-mediated gene transfer system was used to introduce m gamma-IFN and h gamma-IFN genes into mouse and human tumor cells, respectively. Murine tumor cell lines and primary human melanoma tumor cells were successfully transduced with gamma-IFN vector, and these transduced cells secreted measurable levels of biologically active m gamma-IFN and h gamma-IFN, respectively. Both murine and human tumor cell lines that expressed gamma-IFN exhibited increased surface expression of HLA class I antigens when tested by Western blot and FACS analysis. gamma-IFN--transduced human melanoma cells were more active in stimulating tumor-specific cytolytic activity of CTLs from melanoma patients in vitro. m gamma-IFN--transduced tumor cells were substantially less tumorigenic than the corresponding parent tumor cell lines in immune-competent mice. In addition, injection of m gamma-IFN--transduced tumor cells resulted in activation of tumor-specific CTL in vivo. We plan to use gamma-IFN--transduced autologous tumor cells to boost host immune responses as a potential therapy for human melanoma.

L53 ANSWER 20 OF 50 MEDLINE on STN

94214831. PubMed ID: 8162312. Characterization and quality control of autologous gene transfer therapeutics. **Jolly D J**. (Viagene, Inc., San Diego, CA 92121-1204.) Cell transplantation, **(1994)** 3 Suppl 1 S57-60. Ref: 11. Journal code: 9208854. ISSN: 0963-6897. Pub. country: United States. Language: English.

L53 ANSWER 21 OF 50 MEDLINE on STN

93271833. PubMed ID: 1845120. Induction of anti-HIV-1 immune responses by retroviral vectors. **Jolly D J**; Warner J F. (Viagene, Inc., San Diego, California 92121.) Biotechnology therapeutics, **(1991)** 2 (1-2) 179-93. Journal code: 8918082. ISSN: 0898-2848. Pub. country: United States. Language: English.

AB Retroviral vectors encoding HIV-1 proteins, in particular, the envelope from HIV-1 IIIB, have been constructed and used to generate infectious vector particles. Murine cells transduced with these vectors express HIV proteins. Vector-transduced cells, when injected into syngeneic BALB/c mice, induce potent CD8+, class I MHC-restricted cytotoxic T-lymphocyte responses and elicit the production of neutralizing antibody specific for HIV-1. The induction of similar responses in primates may provide the basis for considering the use of these vectors as immunostimulants in humans. The retroviral vectors or vector-transduced cells would probably be first employed as an immunotherapeutic for HIV-infected individuals.

L53 ANSWER 22 OF 50 MEDLINE on STN

93267793. PubMed ID: 8497058. Cross-reactive lysis of human targets infected with prototypic and clinical human immunodeficiency virus type 1 (HIV-1) strains by murine anti-HIV-1 IIIB env-specific cytotoxic T lymphocytes. Chada S; DeJesus C E; Townsend K; Lee W T; Laube L; **Jolly D J**; Chang S M; Warner J F. (Department of Molecular Virology, Viagene Inc., San Diego, California 92121.) Journal of virology, **(1993 Jun)** 67 (6) 3409-17. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB To evaluate the ability of murine anti-human immunodeficiency virus type 1 (HIV-1) IIIB env cytotoxic T lymphocytes (CTL) to recognize and lyse

HIV-1 infected cells, we have constructed a human cell line (Hu/Dd) expressing both the CD4 receptor and the murine H-2Dd major histocompatibility complex (MHC) class I protein. This cell line can be productively infected with HIV-1 and can also function as a target for murine CD8+, class I MHC-restricted CTL directed against the envelope glycoprotein of HIV-1 IIIB. The ability of BALB/c anti-HIV-1 IIIB env CTL to specifically recognize and lyse Hu/Dd target cells infected with divergent HIV-1 strains was tested by using both prototypic and clinical HIV-1 strains. CTL generated by immunization of mice with syngeneic cells expressing either the native or V3 loop-deleted (delta V3) envelope glycoprotein from HIV-1 IIIB were able to recognize and specifically lyse Hu/Dd target cells infected with the HIV-1 prototypic isolates IIIB, MN, WMJ II, SF2, and CC as well as several HIV-1 clinical isolates. These results demonstrate that CTL determinants for HIV-1 env exist outside the hypervariable V3 region, anti-HIV-1 IIIB env CTL appear to recognize common determinants on diverse HIV-1 strains, and classification of HIV-1 strains based on neutralizing antibody reactivities does not appear to correspond to CTL recognition and lysis. The results suggest that the cell-mediated components of the immune system may have a broader recognition of divergent HIV-1 strains than do the humoral components.

L53 ANSWER 23 OF 50 MEDLINE on STN

92182248. PubMed ID: 1966858. Retroviral vectors as vaccines and immunotherapeutics. **Jolly D J**; Warner J F. (Viagene Inc, San Diego, CA 92121.) Seminars in immunology, **(1990 Sep)** 2 (5) 329-39. Ref: 44. Journal code: 9009458. ISSN: 1044-5323. Pub. country: United States. Language: English.

AB Retroviral vectors have been tested in mice for their ability to induce immune response to the proteins which their genomes encode. Using HIV encoded proteins as a model, potent cytotoxic T lymphocyte (CTL) and antibody responses are seen to these proteins. This apparent efficacy of induction may be due to the relative simplicity of the vector and the manner in which antigens are presented to the immune system. Safety issues and other proposed methods of CTL induction are discussed. The potential application of retroviral vectors as immune stimuli in man seems quite broad (viral diseases and cancer), and the first attempted applications in man are likely to be therapeutic rather than prophylactic.

L53 ANSWER 24 OF 50 MEDLINE on STN

92030386. PubMed ID: 1931234. Induction of HIV-specific CTL and antibody responses in mice using retroviral vector-transduced cells. Warner J F; Anderson C G; Laube L; **Jolly D J**; Townsend K; Chada S; St Louis D. (Immunobiology Group, Viagene, Inc., San Diego, CA 92121.) AIDS research and human retroviruses, **(1991 Aug)** 7 (8) 645-55. Journal code: 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB Recombinant retroviral vectors can efficiently transduce and express foreign genes in mammalian cells. We have examined the utility of retroviral vector-mediated gene transfer to deliver genes which encode human immunodeficiency virus type I (HIV) antigens capable of stimulating specific immune responses. Murine fibroblast cell lines were transduced with a nonreplicating murine retroviral vector carrying the gene encoding the HIV-IIIB envelope protein and were shown to express the gp160/120 protein. Mice immunized with syngeneic vector-transduced cells developed CD8+, class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes (CTL) specific for targets expressing the HIV envelope protein. The CTL also exhibited lytic activity on target cells coated with synthetic peptides derived from the gp120 V3 hypervariable region of both the HIV-IIIB and HIV(MN) isolates. Following adoptive transfer in a murine tumor model, these CTL were shown to be effective in vivo by their ability to eliminate established tumor cells expressing the HIV protein. Vector-transduced syngeneic cells were also capable of eliciting HIV envelope-specific antibody responses in immunized mice. Sera obtained from these mice were found to bind to the HIV-IIIB gp160 protein as well as a peptide-defined neutralizing antibody epitope contained within the V3 domain of gp120. These sera exhibited virus-neutralizing activity in that they markedly reduced the ability of HIV to infect and form syncytia of a

human T cell line. This is the first demonstration that cells transduced with a retroviral vector encoding the HIV-IIIB envelope protein are capable of inducing effective HIV-specific cellular and humoral immune responses in mice.

L53 ANSWER 25 OF 50 MEDLINE on STN
92002312. PubMed ID: 1911930. HIV infection and gene transfer therapy. **Jolly D J.** (Viagene, Inc., San Diego, CA 92121.) Human gene therapy, **(1991 Summer)** 2 (2) 111-2. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

L53 ANSWER 26 OF 50 MEDLINE on STN
88093772. PubMed ID: 3480408. High-efficiency gene transfer into cells. **Jolly D J;** Yee J K; Friedmann T. Methods in enzymology, **(1987)** 149 10-25. Journal code: 0212271. ISSN: 0076-6879. Pub. country: United States. Language: English.

L53 ANSWER 27 OF 50 MEDLINE on STN
88092494. PubMed ID: 2826889. Cellular distribution and hormonal regulation of h-SBP in human hepatoma cells. Mercier-Bodard C; Radanyi C; Roux C; Groyer M T; Robel P; Dadoune J P; Petra P H; **Jolly D J;** Baulieu E E. (Lab. Hormones, Inserm U 33, Bicetre, France.) Journal of steroid biochemistry, **(1987)** 27 (1-3) 297-307. Journal code: 0260125. ISSN: 0022-4731. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The cellular distribution of human Sex Steroid Binding Plasma Protein (h-SBP) was studied in human cells and tissues by indirect immunofluorescence. h-SBP was detected in the cytoplasm of hepatocytes, of prostate and epididymis epithelial cells and in endometrium. Sexual and non-sexual skin, intestine epithelium, striated muscle and some rodent organs were not labelled. The intracellular localization of h-SBP indicate that h-SBP could be taken up from the extracellular compartment or synthesized in situ in sex steroid target organs, where it may play a role in hormone uptake. The hormonal regulation of h-SBP secretion by a human hepatoma cell line, H5A, showed that tri-iodothyronine was more potent than estradiol or tamoxifen, which acted as estrogen agonist, in increasing secreted h-SBP and the combined effect of both thyroid and estrogen hormones resulted in an additive stimulation of h-SBP secretion. As shown by Northern blot analysis, oligonucleotides synthesized from the known sequence of h-SBP hybridized with a RNA of approximately 2 kb which was more represented in H5A cells than in normal human liver, and was increased 2-3 times after hormonal stimulation of the cells. The presence of a poly(A+)RNA coding for h-SBP in the human liver indicated the hepatic synthesis of this protein.

L53 ANSWER 28 OF 50 MEDLINE on STN
88087102. PubMed ID: 3335498. A region in the steroid binding domain determines formation of the non-DNA-binding, 9 S glucocorticoid receptor complex. Pratt W B; **Jolly D J;** Pratt D V; Hollenberg S M; Giguere V; Cadepond F M; Schweizer-Groyer G; Catelli M G; Evans R M; Baulieu E E. (Institut National de la Sante et de la Recherche Medicale U33, Universite Paris-Sud, Bicetre, France.) Journal of biological chemistry, **(1988 Jan 5)** 263 (1) 267-73. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB This work was initiated to determine if a specific region of the glucocorticoid receptor determines the formation of the inactive (i.e. non-DNA-binding) 9 S form of the receptor recovered in cytosol preparations. It is known that the murine glucocorticoid receptor of the nti phenotype, which consists of only the carboxyl-terminal 40-kDa peptide containing the DNA-binding and steroid-binding domains separated by a short linker region, is recovered in hypotonic lysates as a 9 S heteromeric complex (Gehring, U., and Arndt, H. (1985) FEBS Lett. 179, 138-142). To further localize the domain required for formation of the 9 S complex, we have determined the sedimentation coefficients of receptors produced in COS-7 cells transfected with several mutants of the human glucocorticoid receptor gene. Deletion of the DNA-binding domain results in a 9 S complex that is somewhat less stable than the wild type receptor

yields a molybdate-stabilized 9 S complex, but deletion of the entire steroid-binding domain or internal deletion of the amino-terminal two-thirds of this domain yields receptors that are constitutive transcriptional activators and are present in cytosol only in the 4 S form. Taken together, these observations demonstrate that the steroid-binding domain contains the features required for formation of the 9 S heteromeric complex, and they are consistent with the proposal that the steroid-binding domain normally represses receptor function.

L53 ANSWER 29 OF 50 MEDLINE on STN
87260956. PubMed ID: 3474647. Gene expression from transcriptionally disabled retroviral vectors. Yee J K; Moores J C; **Jolly D J**; Wolff J A; Respess J G; Friedmann T. Proceedings of the National Academy of Sciences of the United States of America, **(1987 Aug)** 84 (15) 5197-201. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Retroviral vectors are used for the efficient transfer of foreign genes into mammalian cells. We report here the construction of murine retrovirus-based vectors carrying the full-length cDNA for human hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8) and from which the enhancer sequences, the "CAAT box," and the "TATA box" in the long terminal repeats (LTRs) have been deleted. After infection of HPRT-deficient rat cells by the vectors, transcriptional activity from the 5' LTR was undetectable and expression of the HPRT cDNA was dependent on an internal promoter. Removal of the LTR regulatory elements increased HPRT gene expression from an internal promoter, indicating interference between the two sets of transcriptional signals. Such disabled vectors may reduce the likelihood of undesirable genetic changes through insertional mutagenesis in cells infected with retroviral vectors.

L53 ANSWER 30 OF 50 MEDLINE on STN
87248113. PubMed ID: 2439408. Epitope insertion into the human hypoxanthine phosphoribosyltransferase protein and detection of the mutant protein by an anti-peptide antibody. Yee J K; **Jolly D J**; Miller A D; Willis R; Wolff J; Friedmann T. Gene, **(1987)** 53 (1) 97-104. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB The translational stop codon TAA of the human hypoxanthine phosphoribosyltransferase (HPRT) cDNA has been changed to GAA by site-specific mutagenesis. This modification extends the open reading frame to a downstream stop codon and results in the addition of a unique negatively charged hexapeptide to the C terminus of human HPRT protein. The mutated cDNA was transferred into HPRT-deficient rodent cells by retroviral vector infection, and the expressed enzyme was found to be fully active. An antibody against a synthetic octapeptide corresponding to the mutated HPRT C terminus precipitated the HPRT protein specifically from cells infected with the mutant virus and not infected with the wild-type HPRT virus. The technique of inserting a novel epitope into a protein by site-directed mutagenesis should be generally applicable in studies of the regulation of gene expression in vitro and in vivo.

L53 ANSWER 31 OF 50 MEDLINE on STN
87217078. PubMed ID: 3034493. Gene expression from a transcriptionally disabled retroviral vector. Yee J K; **Jolly D J**; Moores J C; Respess J G; Friedmann T. Cold Spring Harbor symposia on quantitative biology, **(1986)** 51 Pt 2 1021-6. Journal code: 1256107. ISSN: 0091-7451. Pub. country: United States. Language: English.

L53 ANSWER 32 OF 50 MEDLINE on STN
87064381. PubMed ID: 3023873. Variable stability of a selectable provirus after retroviral vector gene transfer into human cells. **Jolly D J**; Willis R C; Friedmann T. Molecular and cellular biology, **(1986 Apr)** 6 (4) 1141-7. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Human lymphoblasts deficient in the enzyme hypoxanthine-guanine

phosphoribosyltransferase (HPRT) were infected with an amphotropic helper-free retroviral vector expressing human HPRT cDNA. The stability and expression of the HPRT provirus in five cell lines with different proviral integration sites were examined by determining HPRT mutation and reversion frequencies and by blot hybridization studies. Mutation to the HPRT-negative phenotype occurred at frequencies of approximately 4 X 10(-5) to 3 X 10(-6) per generation. Most mutations in each of the five cell lines were associated with partial or complete deletions or rearrangements of the provirus. Several mutants retained a grossly intact HPRT provirus, and in one such mutant HPRT shutdown resulted from a revertible epigenetic mechanism that was not associated with global changes in proviral methylation. Therefore, mutation and shutdown of the HPRT provirus in human lymphoblasts result from mechanisms similar to those reported for several other avian and mammalian replication-competent retroviruses.

L53 ANSWER 33 OF 50 MEDLINE on STN

87006506. PubMed ID: 2875930. The role of the HPRT gene in human disease. **Jolly D J.** Horizons in biochemistry and biophysics, **(1986)** 8 123-68. Ref: 80. Journal code: 7502793. ISSN: 0096-2708. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Human HPRT deficiency leads to two major forms of human disease. Partial enzyme deficiency results in gouty arthritis, while an almost complete deficiency leads to the Lesch-Nyhan disease. The latter is characterized by severe neurological dysfunction in addition to gouty arthritis, including retardation, choreoathetosis and aggressive and compulsive self-mutilation. The biochemical basis for the neurological symptoms is not understood. The human and mouse cDNA (RNA copy) genes have been isolated and sequenced. In addition, the amino acid sequence of the human protein has been directly determined. The human and mouse proteins differ at 7 amino acids out of the total, (including the N terminal methionine, which is processed off during maturation) of 218. There are 42 out of 654 nucleotide differences between the human and mouse genes in the amino acid coding region. The mouse genomic structure has been determined. It has 9 exons and 8 introns with a total size of approximately 36 kb. The human gene is very similar with identical intron-exon junction points and approximately the same total gene size. Both mouse and human presumed promotor region at the 5' end, lack a recognizable promotor in the form of a "TATAA" box and are very G-C rich, though not the same. This may be a feature of most "housekeeping" genes. HPRT gene point mutations in three gouty arthritis and one Lesch-Nyhan patient have been identified by peptide sequencing. Six gross gene rearrangements have been identified in Lesch-Nyhan HPRT genes. However it is likely that most mutations are point mutations or small deletions. So far all gene mutations identified are different from all others. The gene has been engineered into retrovirus vehicles which allows its efficient introduction into a wide variety of cells, including mouse marrow stem cells. This may allow treatment of Lesch-Nyhan patients as a model of gene therapy.

L53 ANSWER 34 OF 50 MEDLINE on STN

86176788. PubMed ID: 3008106. The organization of the human HPRT gene. Kim S H; Moores J C; David D; Respess J G; **Jolly D J;** Friedmann T. Nucleic acids research, **(1986 Apr 11)** 14 (7) 3103-18. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The organization of the X-linked gene for human hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8.) has been determined by a combination of restriction endonuclease mapping, heteroduplex analysis and DNA sequence analysis of overlapping genomic clones. The entire gene is 42 kilobases in length and split into 9 exons. The sizes of the 7 internal exons and the exon-intron boundaries are identical to those of mouse HPRT gene. The 5' end of the gene lacks the prototypical 5' transcriptional regulatory sequence elements but contains extremely GC-rich sequences and five GC hexanucleotide motifs (5'-GGCGGG-3'). These structural features are very similar to those found in the mouse HPRT gene and to some of the regulatory signals common to a class of constitutively expressed "housekeeping" genes. Several transcriptional start sites have

been identified by nuclease protection studies. Extensive sequence homology between the mouse and human genes is found in the 3' non-coding portion of the gene.

L53 ANSWER 35 OF 50 MEDLINE on STN

86044538. PubMed ID: 3864246. Retroviral vector-mediated gene transfer into human hematopoietic progenitor cells. Gruber H E; Finley K D; Hershberg R M; Katzman S S; Laikind P K; Seegmiller J E; Friedmann T; Yee J K; **Jolly D J**. Science, **(1985 Nov 29)** 230 (4729) 1057-61. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB The transfer of the human gene for hypoxanthine phosphoribosyltransferase (HPRT) into human bone marrow cells was accomplished by use of a retroviral vector. The cells were infected in vitro with a replication-incompetent murine retroviral vector that carried and expressed a mutant HPRT complementary DNA. The infected cells were superinfected with a helper virus and maintained in long-term culture. The production of progeny HPRT virus by the bone marrow cells was demonstrated with a colony formation assay on cultured HPRT-deficient, ouabain-resistant murine fibroblasts. Hematopoietic progenitor cells able to form colonies of granulocytes or macrophages (or both) in semisolid medium in the presence of colony stimulating factor were present in the nonadherent cell population. Colony forming units cloned in agar and subsequently cultured in liquid medium produced progeny HPRT virus, indicating infection of this class of hematopoietic progenitor cell.

L53 ANSWER 36 OF 50 MEDLINE on STN

85004497. PubMed ID: 6541168. Swimming behavior of X and Y human sperm. Sarkar S; **Jolly D J**; Friedmann T; Jones O W. Differentiation; research in biological diversity, **(1984)** 27 (2) 120-5. Journal code: 0401650. ISSN: 0301-4681. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB A laminar-flow fractionation method, developed primarily for removing dead sperm from human semen, was successfully modified to enrich X and Y sperm to 80% purity, and to characterize each enriched fraction for individual swimming behavior. Y-sperm fractions were rapidly detected by fluorescent cytogenetic staining. Subsequently, the degree of enrichment was quantitated with DNA extracted from each sperm fraction probed with a human male-specific recombinant DNA clone. In stationary fluid, X and Y sperm swam in circles with the same average speed. However, in a flowstream, X sperm shifted to a nearly straight path of movement in a significantly decreased angular velocity. This shift was four times more pronounced in X sperm than in Y sperm, especially after the initial transition from stationary fluid to flow. The velocity gradient across the flow axis was essential for separating X and Y sperm; uniform flow velocity did not separate them effectively.

L53 ANSWER 37 OF 50 MEDLINE on STN

84250222. PubMed ID: 6377498. Expression of a retrovirus encoding human HPRT in mice. Miller A D; Eckner R J; **Jolly D J**; Friedmann T; Verma I M. Science, **(1984 Aug 10)** 225 (4662) 630-2. Journal code: 0404511. ISSN: 0036-8075. Pub. country: United States. Language: English.

AB Transmissible retroviruses encoding human hypoxanthine phosphoribosyltransferase (HPRT) were used to infect mouse bone marrow cells in vitro, and the infected cells were transplanted into mice. Both active human HPRT-protein and chronic HPRT-virus production were detected in hematopoietic tissue of the mice, showing transfer of the gene. These results indicate the possible use of retroviruses for somatic cell therapy.

L53 ANSWER 38 OF 50 MEDLINE on STN

84239730. PubMed ID: 6203897. Partial phenotypic correction of human Lesch-Nyhan (hypoxanthine-guanine phosphoribosyltransferase-deficient) lymphoblasts with a transmissible retroviral vector. Willis R C; **Jolly D J**; Miller A D; Plent M M; Esty A C; Anderson P J; Chang H C; Jones O W; Seegmiller J E; Friedmann T. Journal of biological chemistry, **(1984 Jun**

country: United States. Language: English.

AB A human Lesch-Nyhan (hereditary, severe hypoxanthine-guanine phosphoribosyltransferase (HPR transferase) deficiency) B-lymphoblast line was infected with an amphotropic retroviral vector containing human HPR transferase cDNA under transcriptional control of viral long terminal repeat sequences. Of 17 clones isolated, 12 integration groups were defined by analysis of restriction enzyme digests of their genomic DNA with HPR transferase and viral long terminal repeat probes. These groups had HPR transferase activity restored to levels of 4 to 23% of normal values. Aberrant metabolic parameters associated with severe deficiency of HPR transferase activity, i.e. elevated rates of purine excretion, increased accumulation of hypoxanthine, elevated 5-phosphoribosyl-1-pyrophosphate contents, altered nucleoside triphosphate pools, resistance to toxic effects of 6-thioguanine, were partially to nearly completely corrected; the degree of correction generally corresponded to the degree of restoration of HPR transferase activity. The integration of the HPR transferase gene was found to be variably stable during 9 months of culture of the virally transformed lymphoblasts under nonselective conditions. The HPR transferase gene-infected lines reverted to resistance to 20 microM 6-thioguanine, i.e. severe HPR transferase deficiency, at frequencies of 10(-6) to in excess of 10(-5) per generation. The reversions were accompanied by either a loss or rearrangement of the integrated HPR transferase sequences or by retention of the sequences in an unaltered form.

L53 ANSWER 39 OF 50 MEDLINE on STN

84194094. PubMed ID: 6585829. Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: implications for X-chromosome inactivation. Wolf S F; **Jolly D J**; Lunnen K D; Friedmann T; Migeon B R. Proceedings of the National Academy of Sciences of the United States of America, **(1984 May)** 81 (9) 2806-10. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB To explore the role of DNA methylation in maintaining dosage compensation of X chromosome-linked genes and in regulating the transcriptional activity of "housekeeping" genes, we characterized DNA methylation of active, inactive, and derepressed alleles at the locus for hypoxanthine phosphoribosyltransferase (HPRT) on the human X chromosome. The methylation of Hpa II and Hha I sites in HPRT alleles on the active X chromosome was the same in all tissues. The consensus pattern includes hypomethylation of 5' clustered sites and extensive methylation of the 3' sequence. The striking feature of methylation of inactive X-chromosome alleles is nonuniformity and less extensive hypomethylation of the 5' cluster. Analysis of HPRT alleles reactivated in response to 5-azacytidine showed at least partial restoration of the consensus pattern. These observations indicate that methylation of housekeeping genes on the X chromosome is the same as that of autosomal ones and that the overall pattern and methylation of multiple sites within a cluster may cooperate to facilitate transcription. Furthermore, the fidelity of methylation of the active allele and the extensive drift in methylation of the inactive allele suggest that mechanisms involved in X-chromosome dosage compensation may be directed at the active rather than inactive X chromosome.

L53 ANSWER 40 OF 50 MEDLINE on STN

83273685. PubMed ID: 6308645. A transmissible retrovirus expressing human hypoxanthine phosphoribosyltransferase (HPRT): gene transfer into cells obtained from humans deficient in HPRT. Miller A D; **Jolly D J**; Friedmann T; Verma I M. Proceedings of the National Academy of Sciences of the United States of America, **(1983 Aug)** 80 (15) 4709-13. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB A cDNA corresponding to the human gene for hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) has been ligated into murine retroviral vectors such that it is under the transcriptional control of

viral long terminal repeats. Transfection of HPRT- cells followed by superinfection with various helper viruses has led to the rescue of chimeric virus capable of transmitting the HPRT+ phenotype to HPRT- rodent or human cells. These genetically transformed cells contain authentic human HPRT at levels similar to normal HPRT+ cells.

L53 ANSWER 41 OF 50 MEDLINE on STN

83169681. PubMed ID: 6300847. Isolation and characterization of a full-length expressible cDNA for human hypoxanthine phosphoribosyl transferase. **Jolly D J**; Okayama H; Berg P; Esty A C; Filpula D; Bohlen P; Johnson G G; Shively J E; Hunkapillar T; Friedmann T. Proceedings of the National Academy of Sciences of the United States of America, **(1983 Jan)** 80 (2) 477-81. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have cloned a full-length 1.6-kilobase cDNA of a human mRNA coding for hypoxanthine phosphoribosyltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8) into a simian virus 40-based expression vector and have determined its full nucleotide sequence. The inferred amino acid sequence agrees with a partial amino acid sequence determined for authentic human HPRT protein. Transfection of HPRT-deficient mouse LA9 cells with the purified plasmid leads to the expression of human HPRT enzyme activity in cells stably transfected and selected for enzyme activity in hypoxanthine/aminopterin/thymidine medium.

L53 ANSWER 42 OF 50 MEDLINE on STN

83168926. PubMed ID: 6300781. Elements in the long terminal repeat of murine retroviruses enhance stable transformation by thymidine kinase gene. **Jolly D J**; Esty A C; Subramani S; Friedmann T; Verma I M. Nucleic acids research, **(1983 Mar 25)** 11 (6) 1855-72. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We have investigated the effects of long terminal repeats (LTRs) of murine retroviruses on the frequency of obtaining stable transfectants by the herpes virus thymidine kinase (TK) gene. The results indicate that addition of LTRs enhances the number of TK+ transformants by 10-20 fold. A 5-12 fold enhancement was also observed when chromosomal DNA from either human or hamster cells was mixed with a plasmid containing LTR sequences and transfected onto LTR- cells. The LTR sequences involved in the enhancement were localized in the region which contains tandem repeats. All other regions of the LTR did not show any enhancement of stable TK+ transfectants. The location or the orientation of the enhancer sequences with respect to the TK gene did not exert any influence on the frequency of transformation. The enhancement effect does not appear to be linked to either increased numbers of chromosomal integrations or elevated levels of transcription of the TK gene.

L53 ANSWER 43 OF 50 MEDLINE on STN

83015027. PubMed ID: 6956912. Isolation of a genomic clone partially encoding human hypoxanthine phosphoribosyltransferase. **Jolly D J**; Esty A C; Bernard H U; Friedmann T. Proceedings of the National Academy of Sciences of the United States of America, **(1982 Aug)** 79 (16) 5038-41. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Mouse cells deficient in the enzyme hypoxanthine phosphoribosyltransferase (HPRT; EC 2.4.2.8) have been transfected with total human DNA, and cells producing human enzyme were isolated by growth in selective medium. DNA from several such cell lines has been used to generate secondary transfectants that make human HPRT. Blots of the DNA of these secondary cells have been hybridized with total human DNA probes or with cloned human Alu sequences, and one of several common bands has been cloned in pBR322. Colonies of transformed Escherichia coli containing human sequences were detected by their homology with human DNA, and subclones of resulting recombinant plasmids were prepared. Two subclones free of Alu sequences were found to contain human sequences that hybridized to human X chromosome DNA. One of these, pBR1.5, also hybridized to a single RNA band on gel blots of human and secondary transfectant cytoplasmic poly(A)+RNA but not to RNA from the parent mouse cell line. These results

indicate that these clones represent human HPRT gene fragments. This has been confirmed by using pBR1.5 as a probe to isolate an authentic and expressible human HPRT cDNA clone from a library prepared by H. Okayama and P. Berg.

L53 ANSWER 44 OF 50 MEDLINE on STN
82122574. PubMed ID: 6276559. Base sequence studies of 300 nucleotide renatured repeated human DNA clones. Deininger P L; **Jolly D J**; Rubin C M; Friedmann T; Schmid C W. Journal of molecular biology, **(1981 Sep 5)** 151 (1) 17-33. Journal code: 2985088R. ISSN: 0022-2836. Pub. country: ENGLAND: United Kingdom. Language: English.

L53 ANSWER 45 OF 50 MEDLINE on STN
80122222. PubMed ID: 6243780. Nuclear RNA transcripts from Drosophila melanogaster ribosomal RNA genes containing introns. **Jolly D J**; Thomas C A Jr. Nucleic acids research, **(1980 Jan 11)** 8 (1) 67-84. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The transcription of ribosomal genes in a cell line (Kc) derived from female D.melanogaster, has been investigated using hybridization probes prepared from restriction fragments of a cloned rDNA repeat with a 5 kb type I [Wellauer et al. (1978) Cell 14, 269-278] intron. Gels, of nuclear RNA that have been transferred to diazotized paper and hybridized with labelled intron sequences, reveal both large (1-10 kb) transcripts and a discrete 325 base species. Berk-Sharp experiments [(1977) Cell 12, 721-732] reveal large transcripts that are homologous to intron sequences and extend into 28S sequences as well. However, while the abundance of 28S transcripts is about 50,000 copies per nucleus [Clark et al. (1977) Genetics 86, 789-800], these long transcripts are only present at 1-2 copies per nucleus and the 325 base species is only 10 times more abundant. In view of the fact that female cells have about 400 rDNA genes, 49% of which have type I introns, one must conclude either that transcription rarely occurs from the genes containing introns (the majority) or these transcripts are processed unusually rapidly. Transcripts homologous to the "non-transcribed spacer" region have been found, but their abundance is no higher.

L53 ANSWER 46 OF 50 MEDLINE on STN
73233114. PubMed ID: 4723772. Light-scattering studies on supercoil unwinding. Campbell A M; **Jolly D J**. Biochemical journal, **(1973 Jun)** 133 (2) 209-26. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

L53 ANSWER 47 OF 50 MEDLINE on STN
73161196. PubMed ID: 4658976. Tertiary-structure transitions of supercoiled deoxyribonucleic acid. **Jolly D J**; Campbell A M. Biochemical journal, **(1972 Sep)** 129 (3) 42P. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

L53 ANSWER 48 OF 50 MEDLINE on STN
73144364. PubMed ID: 4656791. Light-scattering studies on deoxyribonucleic acid flexibility. The solution properties of a small circular deoxyribonucleic acid molecule. **Jolly D J**; Campbell A M. Biochemical journal, **(1972 Dec)** 130 (4) 1019-28. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

L53 ANSWER 49 OF 50 MEDLINE on STN
73045299. PubMed ID: 4634829. The three-dimensional structure of supercoiled deoxyribonucleic acid in solution. Evidence obtained from the angular distribution of scattered light. **Jolly D J**; Campbell A M. Biochemical journal, **(1972 Jul)** 128 (3) 569-78. Journal code: 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

L53 ANSWER 50 OF 50 MEDLINE on STN
73015598. PubMed ID: 5076667. Resolution of supercoiled deoxyribonucleic acid structures by light-scattering. Campbell A M; **Jolly D J**.

2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom.
Language: English.

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(FILE 'HOME' ENTERED AT 19:50:08 ON 09 MAR 2004)

FILE 'USPATFULL' ENTERED AT 19:50:29 ON 09 MAR 2004
E SANDERS DAVID A/IN
L1 2 S E3 OR E4
E FISCHBACH MICHAEL A/IN
L2 1 S E4
E KUHN RICHARD J/IN
L3 2 S E3
E JEFFERS SCOTT A/IN
L4 1 S E3
E NORTH CYNTHIA L/IN

FILE 'MEDLINE' ENTERED AT 19:52:30 ON 09 MAR 2004
E SANDERS D A/AU
L5 245 S E2 OR E3
L6 6 S L5 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
E FISCHBACH M A/AU
L7 134 S E2
L8 2 S L7 AND (RETROVIR? OR EXPRESSION VECTOR? OR ROSS RIVER VIRUS O
L9 2 S L8 NOT L6
E KUHN R J/AU
L10 95 S E3
L11 9 S L10 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
L12 8 S L11 NOT (L6 OR L9)
E JEFFERS S A/AU
L13 33 S E2 OR E5
L14 2 S L13 AND (RETROVIR? OR EXPRESSION VECTOR? OR PSEUDOTYP? OR ROS
E NORTH C L/AU
L15 10 S E3

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E SANDERS D A/IN
L16 4 S E3
E FISCHBACH M A/IN
L17 1 S E3
E KUHN R J/IN
L18 2 S E3
E JEFFERS S A/IN
L19 3 S E3
E NORTH C L/IN
L20 1 S E3

FILE 'MEDLINE' ENTERED AT 20:05:31 ON 09 MAR 2004

FILE 'USPATFULL' ENTERED AT 20:05:42 ON 09 MAR 2004
L21 13358 S (RETROVIR? VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEUDOT
L22 2617 S L21 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L23 106 S L22 AND (MOMLV/CLM OR MOLONEY MURINE LEUKEMIA VIRUS/CLM)
L24 28 S L23 AND (GAG/CLM OR POL/CLM OR PRO/CLM)
L25 21 S L24 AND AY<2000
L26 12 S L25 AND (MARKER?/CLM)
L27 10 S L26 AND (SELECTABLE/CLM OR DETECTABLE/CLM)
L28 689 S L21 AND (LENTIVIR?/CLM OR FIV/CLM OR HIV/CLM OR SIV/CLM OR BI
L29 5 S L28 AND (LENTIVIR? EXPRESSION VECTOR/CLM)
L30 283 S L28 AND AY<2000
L31 80 S L30 AND (GAG/CLM OR PRO/CLM OR POL/CLM)
L32 16 S L31 AND (MARKER?/CLM)
L33 0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)

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L34           0 S L30 AND (LENTIVIRAL EXPRESSION VECTOR?/CLM)
L35          10 S LENTIVIRAL EXPRESSION VECTOR?
L36          15 S L30 AND (LENTIVIR? VECTOR?/CLM)
L37           3 S L36 AND MARKER?/CLM

     FILE 'MEDLINE' ENTERED AT 20:27:55 ON 09 MAR 2004
L38      123851 S (RETROVIR? OR VECTOR? OR RETROVIR? EXPRESSION VECTOR? OR PSEU
L39        1703 S L38 AND (MOMLV OR MOLONEY MURINE LEUKEMIA VIRUS)
L40          85 S L39 AND (GAG AND POL)
L41           0 S L40 AND (MULTIVALENT)
L42           7 S L40 AND MARKER?
L43          67 S L40 AND PY<2000
L44         602 S L38 AND (LENTIVIR? EXPRESSION VECTOR? OR LENTIVIR? VECTOR?)
L45          65 S L44 AND PY<2000
L46           8 S L45 AND (MARKER? OR SELECTABLE OR DETECTABLE)
L47         269 S (ROSS RIVER VIRUS OR ROSS RIVER ALPHAVIRUS)
L48          39 S L47 AND (ENV?)
L49          23 S L48 AND PY<1999
                E RICHMAN D/AU
                E RICHMAN DOUGLAS ?/AU
L50          21 S E4
                E JOLLY DOUGLAS ?/AU
L51           2 S E4
                E JOLLY D J/AU
L52          68 S E3
L53          50 S L52 AND PY<1999

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ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
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